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CONTENTS

PAGE

AMPLIFICATION OF ACTION CURRENTS WITH THE ELECTRON TUBE IN RECORDING WITH THE STRING GALVANOMETER. *Alexander Forbes and Catharine Thacher* 409

VARIATIONS IN THE RESPIRATORY DEAD AIR SPACE DUE TO CHANGES IN THE DEPTH OF BREATHING. *R. G. Pearce and D. H. Hoover* 472

THE SUB-ARACHNOID AND INTRA-ARTERIAL ADMINISTRATION OF SODIUM BICARBONATE AND OTHER ELECTROLYTES. *J. B. Collip* 483

THE INFLUENCE OF INTERNAL SECRETIONS ON BLOOD PRESSURE AND THE FORMATION OF BILE. *Ardrey W. Downs* 498

GASTRIN STUDIES. V. CHEMICAL STUDIES ON GASTRIN BODIES. *F. C. Koch, A. B. Luckhardt and R. W. Keeton* 508

ESSENTIALS IN MEASURING EPINEPHRIN OUTPUT WITH FURTHER OBSERVATIONS ON ITS RELATION TO THE RATE OF THE DENERVATED HEART. *G. N. Stewart and J. M. Rogoff* 521

THE EFFECT OF SPLENECTOMY UPON GROWTH IN THE YOUNG. *Samuel Chester Henn* 562

THE IMPORTANCE OF VAGAL AND SPLANCHNIC AFFERENT IMPULSES ON THE ONSET AND COURSE OF TETANIA PARATHYROPRIVA. *Walter Lincoln Palmer* 581

INDEX 591

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AMPLIFICATION OF ACTION CURRENTS WITH THE ELECTRON TUBE IN RECORDING WITH THE STRING GALVANOMETER[1]

ALEXANDER FORBES AND CATHARINE THACHER

From the Laboratory of Physiology in the Harvard Medical School

Received for publication April 6, 1920

INTRODUCTION

In the spring of 1916 Dr. H. B. Williams suggested the use of the electron tube to amplify those action currents in the nervous system which are too small to record with the string galvanometer alone. In the course of several researches in this laboratory we have encountered difficulties due to the extremely small excursions obtainable with the string galvanometer from the action currents in nervous tissue, notably in the activity of the spinal cord and in the motor nerve involved in the crossed extension reflex. The suggestion appeared therefore to offer possibilities of attacking physiological problems hitherto inaccessible.

Military duties prevented the development of this suggestion until the spring of 1919, when conditions in this laboratory became favorable for the work. Doctor Williams was still absent from his laboratory on duty; but the prospect of utility of the method made us feel justified in proceeding to develop it without waiting for his return and the collaboration we should have preferred. We wish to express our appreciation of his generosity in urging us to publish the work as our own, and to thank him for certain helpful suggestions in the later phases of the work since he returned to his laboratory.

There is nothing new in the idea of amplifying electric currents with the electron tube. Especially in the field of radio communica-

[1] A preliminary report has appeared; This Journal, 1920, li, 177 (proceedings).

tion, this has been standard practice for years. The usual method of observing the currents amplified by this device is with the telephone receiver. This method is not applicable to those problems in physiology which have led us to this work. In using the string galvanometer to record the amplified currents we are confronted with several new problems which do not enter into the telephone method. These problems are of practical importance to the physiologist who would use the method, and of some theoretical interest to the physicist. It has seemed worth while to carry out a good many experiments and to attempt to correlate them with theoretical expectation, in order to formulate, if possible, the correct method of obtaining the maximum amplification, or at least to outline the principles on which such a method must depend. We have therefore taken up some of the mathematical considerations involved although these may be of little interest to the physiologist who wishes merely to know how to use the method. The mathematical discussion may readily be skipped, and simple instructions still be found for obtaining very nearly the maximum possible amplification.

THE ELECTRON TUBE

The electron tube or thermionic amplifier (1), commonly known in this country as the audion, consists essentially of a vacuum tube containing a cathode in the form of a filament, heated to incandescense by the passage of a current, an anode in the form of a metal plate, and an auxiliary electrode in the form of a grid (2) placed between the anode and the cathode. A conventional and schematic representation of these elements is shown in figure 1; this is convenient for identification of the parts in circuital diagrams to follow.

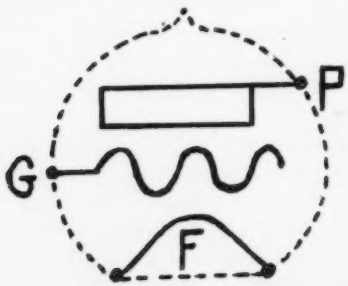


Fig. 1. Conventional diagram of electron tube elements. *F*, filament; *G*, grid; *P*, plate.

When the filament (cathode) is incandescent it emits electrons into the space within the tube. If by means of a battery the plate (anode) is made positive with respect to the filament, these electrons under the influence of the electric field thus set up, travel to the plate and thus enable a current to flow from plate to filament within the

tube (according to the conventional designation of the direction of current flow). Under ordinary conditions of operation, more electrons are emitted by the filament than can be drawn to the plate by the voltage applied to it; a negative "space charge" results which repels electrons subsequently emitted and causes them to return to the filament. This space charge exerts a limiting effect on the current that passes from anode to cathode. If now a positive potential, with respect to filament, be applied to the grid, or auxiliary electrode, it will oppose the space charge and enable more current to flow in the tube; if a negative charge be applied to the grid it will still further limit the current. In this way the current in the tube may be regulated by variations in the potential of the grid. In a properly designed tube small variations in the potential of the grid may be made to produce large variations in the plate-to-filament current, and in this way the device acts as an amplifying relay, the energy of amplification being supplied by the battery which maintains the high potential of the plate.

METHODS IN GENERAL

The salient feature of our problem as compared with the ordinary uses of the electron tube was the necessity of protecting the delicate string of the galvanometer from the direct current used to maintain the high potential of the plate.

Telephone receivers are ordinarily placed directly in series with the plate and the high voltage battery, as is shown in figure 2. This would be impossible with a sensitive string galvanometer. With the Western Electric "D-tube," for instance, the optimum plate potential is 200 volts; under the most favorable working conditions this tube has a plate-to-filament resistance of about 100,000 ohms. Neither string nor telephones placed in series with this would add greatly to the total resistance of the circuit. Therefore, the current that would pass through the string would amount to about 2 milliamperes. One hundredth of this current is as much as or more than it is safe to pass through a string of 2 or 3 micra diameter, the size best suited to nerve physiology. Even were it possible to pass the full plate current through the string without destroying it, to work under this condition would be impossible, for such a current would move the string well out of the field accessible to the beam of light required for projection.

Three methods have presented themselves for utilizing the amplifying energy of the plate battery without placing the string directly in the path of a damaging current. The first is to place the primary coil of a transformer in the plate circuit, and connect the galvanometer across the terminals of the secondary coil of the transformer (see

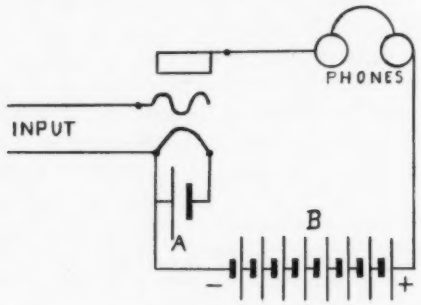


Fig. 2. Typical arrangement of electron tube and circuits commonly used for amplifying currents and detecting them with telephone receivers. *A*, filament-heating battery; *B*, plate battery.

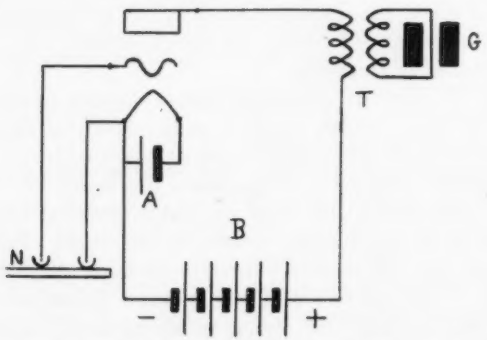


Fig. 3. Transformer method (see text). *N*, nerve or other tissue giving rise to action current. *T*, transformer. *G*, string galvanometer.

fig. 3). The second method was proposed by Mr. S. W. Dean, and is based on the principle of the Wheatstone bridge (fig. 4). It may be designated the bridge method. The third method was proposed by Mr. Sewall Cabot. It consists in protecting the string galvanometer from the direct current by placing it in series with a condenser, and

shunting the plate current by the string and condenser through a resistance of the same order of magnitude as that of the tube (fig. 5).[2] Whittemore (3) has described a scheme for measuring with a string galvanometer radio signals rectified by an electron tube. This procedure, though intended for a wholly different purpose from ours, has much in

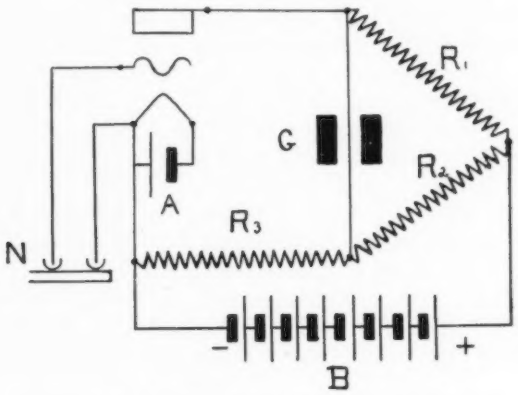


Fig. 4. Bridge method (see text).

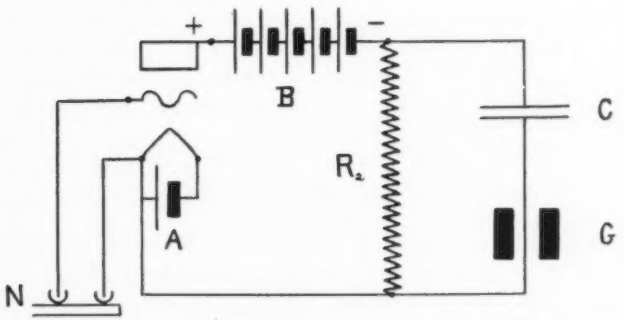


Fig. 5. Condenser method. C, condenser. R_2, by-pass resistance (see text).

[2] This method was first tried with the plate battery between the filament and the galvanometer, etc. At the suggestion of Prof. G. W. Pierce, the battery was shifted to the location shown, next to the plate. It makes no difference in the amplification, but it is better practice to have the string on the low potential side of the circuit, and makes it possible to keep the magnet core of the galvanometer grounded.

common with both the bridge method and the condenser method here described.

The transformer method offers the objection that what is recorded is the *rate of change* of the difference of potential to be amplified; a sustained e. m. f. would give no evidence of its presence by this method, except at its beginning and ending. However, as the action current of a nerve-trunk following a single stimulus is of very rapid onset and brief duration,—so brief that no string has time to attain its full excursion before the maximum e. m. f. is over (4, p. 145)—there might be some use in obtaining records that would show a rate of change. It might serve for the comparison of the magnitudes of successive responses in nerve under conditions insuring constancy of time relations. The method seemed at least worth a trial.

The bridge method consists in causing the current from the high voltage (plate) battery to pass through a split circuit, one branch consisting of R_1 and the tube in series, the other consisting of R_2 and R_3 in series (fig. 4). The string connects the two circuits at their middle points. The best results would be obtained with R_1, R_2 and R_3 all approximately equal to the resistance of the tube, R_b (plate-to-filament), and in any case the proportion $\frac{R_2}{R_3} = \frac{R_1}{R_b}$ must hold.

This method depends on the balance in the bridge, i.e., the equalizing of potential at the two ends of the string. Starting with such a balance, a change in the input difference of potential (between filament and grid) would, by causing a change in the resistance of the tube (plate-to-filament), unbalance the system and create a difference of potential between the ends of the string.

The chief advantage of this method is that changes in the input difference of potential are recorded without distortion; that is, a constant e. m. f. is shown as such in the record.

The chief drawback lies in the difficulty of obtaining a perfect balance, and of maintaining it through an experiment. Another objection is that the resistance of any string commonly in use is so low, compared with those in the bridge, that it approximates a short-circuit and tends to reduce the difference of potential set up between the two points it connects. This tendency will reduce the resulting amplification.

The condenser method, suggested by Cabot, obviates both of these difficulties. There being no current permitted to pass as long as the tube resistance is steady, on account of the interposed condenser, the

problem of balancing does not arise. There is also no such reducing tendency as was mentioned above. The principle of the method is in the main the same. It depends on the fact that if a constant voltage drop occurs through two resistances in series, when one of these changes (the other remaining constant), the voltage drop through it must change; consequently, a change of potential occurs at the end of the string connected to the junctional point where the two resistances are connected.

At first sight it would appear that the condenser would lay this method open to the same objection that applies to the transformer method, that it would distort the record, showing only a rate of change in the input, and failing to record a sustained e. m. f. as such. This objection can readily be overcome for practical purposes by making the condenser very large, so that it provides a virtually constant reservoir of energy throughout the time that an action current lasts. We are chiefly concerned with the action currents of nerves, and these are of brief duration, most of the effect being over in less than 0.01 second; action currents of cardiac or skeletal muscle rarely require amplification. The resistance through which the condenser has to discharge itself with this arrangement under optimum conditions amounts to about 80,000 ohms. Through such a resistance a condenser of 25 mf. requires considerably over 2 seconds for approximately complete discharge; in 0.01 second it would lose 0.5 per cent of its charge. In short, for recording an electrical disturbance lasting only 0.01 second, the method would not introduce any appreciable distortion with a condenser of this order of magnitude. In the case of action currents of skeletal muscle, although their duration is much greater than those of nerve, the distortion is still insignificant. When currents of longer duration are studied, it is usually possible to obtain all the amplification that is needed by slacking the tension on the string. Electron tube amplification, therefore, has its chief use in studying brief and rapidly changing electrical disturbances which a slack string will not follow satisfactorily.

APPARATUS

The electron tubes used in this research were generously loaned us by the Western Electric Company. On the recommendation of Dr. H. B. Arnold "D-tubes" and "L-tubes" were tried, two of each being furnished, with a socket to fit them.

A transformer was also loaned by the Western Electric Company to enable us to try the first-mentioned method. It was an autotransformer with a closed iron core. The windings were in four sections, two of 12.5 ohms each and two of 62.5 ohms each, making a total resistance of 150 ohms. The condenser used in the third method was made of the common Western Electric Company paper condensers of 1 microfarad each, connected in parallel. In nearly all of the experiments 15 were used, giving thus a capacity of 15 mf.

The current for heating the filament was supplied by an Edison storage battery of five type B-2 cells, having a capacity of 40 amperehours. This is conventionally known as the "A-battery." The current was regulated by a small circular rheostat on a porcelain base, such as is commonly used for this purpose. The battery which supplied the plate current (conventionally known as the B-battery) consisted during the experimenal stage of the work chiefly of the small dry cells used for flash-lights, connected in series. This was supplemented by a 24-cell lead storage battery loaned by the Cruft Laboratory of the Harvard Physics Department. When the apparatus was finally arranged as a permanent installation a B-battery of lead storage cells was made in the following way:

A lead strip 1 inch wide by $\frac{1}{16}$ inch thick was passed through a press at the Jefferson Physical Laboratory, thus impressing on it a pattern which increased the surface area; then the strip was cut into suitable lengths. A disc-shaped mould was prepared with two slits through which the lead strips fitted, and with a wire nail protruding upward through the bottom. Two lead strips were passed through the slits to the proper distance and a string tied round them within the mould, then a short piece of glass tubing was put over the wire nail. Then a mixture of paraffin, beeswax and rosin was poured into the mould and allowed to harden, the string serving to strengthen the wax, and the glass tube providing a vent. Small glass jars about 2 inches by 6 were filled with sulphuric acid, and the wax discs with the lead strips protruding downward were fitted into the necks and the openings sealed with hot wax, all but the vents. A wooden separator was placed between the plates of each cell. One hundred and thirty-five of these cells were made and set up in three trays. Each tray had 45 cells connected in series. In order to form the plates the three trays were connected in parallel and charged from the 110-volt mains first in one direction and then in the other. The charging current proved to be so small that to hasten the process storage batteries were con-

nected in series with the 110-volt mains, giving a total charging voltage of 130. When the plates were formed the batteries were connected with a group of three switches so wired that by throwing them all one way the three groups of cells were arranged in series and connected with the system, and by throwing them all the other way the three groups were arranged in parallel and connected with wires whereby they could be charged from the 110-volt power mains. This switching arrangement is included in the general wiring plan shown in figure 17.

The galvanometer used was an Einthoven String Galvanometer furnished by the Cambridge Scientific Instrument Company of Cambridge, England; the same that has been used in this laboratory for several years. In most of the experiments a comparatively coarse platinum string was used, having a diameter of 5 micra and a resistance of 910 ohms (designated string *B*); in some experiments we used a gilded quartz string, furnished by Hindle, of 2.5 micra diameter and 20,000 ohms resistance (string *G*); in the last experiments we used a similar Hindle string of 12,000 ohms.

In order to protect the string during the establishment of the high voltage circuit, and in preliminary tests, a shunt was connected across the terminals of the double-pole switch used to connect the string with all other circuits. For this purpose we used a rheostat of the same type as that used to control the filament current.

For projection we used an arc lamp furnished by A. T. Thompson. As usual, a cylindrical lens was used to focus the beam of light in a horizontal line across the film.

For recording we at first attempted to use a camera in which the film was moved by means of a Sandstrom electric kymograph. This was abandoned for a reason which will be discussed later, and all experiments were performed with a newly constructed camera, used for the first time in this research. Since this camera has proved exceedingly useful and convenient for a wide range of physiological work, and has not yet been described, it is thought worth while to include here a brief description, especially since one feature of it proved essential to success under the conditions met with in these experiments. The camera was designed to make records on standard perforated moving picture film, the motion of which is regulated by a sprocket such as is used in moving picture cameras and projectors. The sprocket is so placed behind the illuminated slit that it presents the surface of the film at the focal plane of a cylindrical lens set in an adjustable holder in the path of the beam of light. It is mounted

on a shaft provided with a conical clutch whereby it may be rapidly started and stopped, the clutch connecting it with a friction drive which is run by an electric motor. The motor is a 12-volt D. C. macine intended for an automobile self-starter, and is completely encased in metal; it runs at 460 r. p. m. This motor turns the disc of the friction drive, which is of cast iron and has a diameter of 32 cm. A spring on the shaft holds this disc firmly in contact with a wheel with a leather rim 18.5 cm. in diameter mounted on the horizontal shaft which

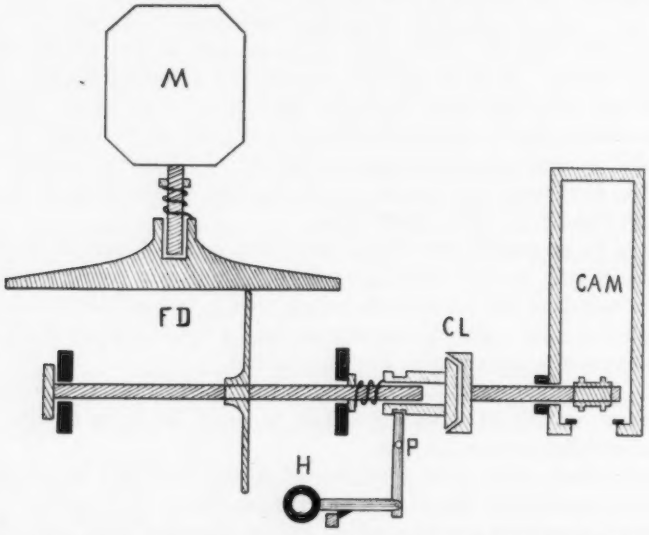


Fig. 6. Horizontal section of photographic recording apparatus at level of optical axis. *M*, motor. *FD*, friction drive. *CL*, clutch. *H*, handle for operating clutch, showing spring, lever pivoted at *P*, and catch. *Cam*, camera.

carries the clutch, and is perpendicular to the motor shaft. The wheel may be moved along the shaft from the center to the rim of the disc, and secured in any desired position with a set-screw, thus providing a wide range of gear ratios (fig. 6). In this way any desired speed of film is obtained, from a minimum of about 7 cm. per second to a maximum of 48 cm. per second. Still slower speeds could be had by putting a resistance in series with the armature of the motor. The film, which comes in 200-foot rolls, is placed in a light-proof magazine of a type used in some moving picture cameras: in this magazine it may be in-

serted in the camera in full daylight. The end of the film is led around the sprocket and into a receiving magazine so arranged that the spool on which it is received is coupled to the shaft of a spring clock-work device which serves to wind up the film as fast as it leaves the sprocket (fig. 7). The lever which operates the clutch also moves a screen which cuts off the beam of light when the film is stopped. This prevents fogging of the film during short intervals between observations. This fogging spoils the film for about an inch on each side of the point behind the slit; on a series of brief observations at a slow speed of film the percentage of waste would be considerable without this screen. The current which drives the motor is furnished by a battery of Gould storage cells of large capacity, which maintains practically constant speed.

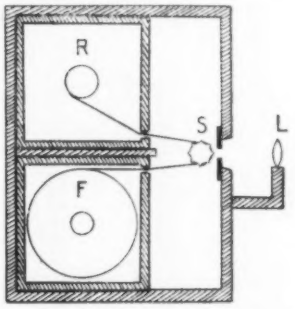


Fig. 7. Vertical section of camera. *F*, feeding spool in magazine. *R*, receiving magazine. *S*, sprocket. *L*, cylindrical lens.

This camera has several advantages over one previously described (4, p. 127) and used hitherto in recording with this galvanometer. It carries 200 feet of film at one loading, as compared with 50 feet, which was practically the maximum capacity of the old camera; and it can be loaded by transferring the magazine to the dark room without moving the camera, which is large and heavy. The old camera had only certain fixed speeds depending on the gear ratios of the Sandstrom kymograph. The friction drive makes it possible to select any speed within the limits mentioned. The most important advantage is the unfailing uniformity of speed imparted to the film by the sprocket engaging in the perforations. It was the great difficulty in obtaining uniform motion with a film pressed against a revolving cylinder by rubber rollers, that led to the abandonment of the old camera. In this particular research an unexpected and indispensable advantage was found in the new camera; this has already been alluded to, and will be discussed later. The chief limitation of the new camera lies in the narrowness of the film (35 mm.), this being the maximum width made with perforations. This difficulty can be overcome by moving the camera nearer to the galvanometer and thus decreasing the magnification. Sharper definition of the image results, and greater contrast in the film

at high speeds. If it is desired to reproduce the record on a larger scale it may easily be enlarged, and the increased definition and contrast will facilitate the process.

ELEMENTARY EXPERIMENTS

For comparison of the results obtained with and without the electron tube, we used in general an artificial source of current to be amplified, instead of the action current of a nerve or muscle. This had the great advantage of saving time, for any desired electromotive force could be drawn at a moment's notice without the labor of dissecting a nerve, placing it in a moist chamber and connecting it with

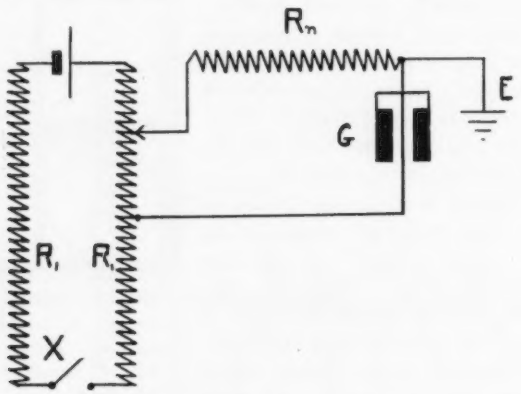


Fig. 8. Standard wiring diagram (simplified). R_1, resistance in primary circuit. X, key in primary circuit. R_n, resistance representing resistance of tissue in series with galvanometer, G. E, connection to earth.

non-polarizable electrodes. Even greater advantages were the dependable uniformity of the source of current and the ease with which its quantity and duration could be regulated, conditions impossible of fulfillment with living tissues. The source of current was an Edison cell connected with a resistance of 640 ohms, R_1, in series with a slide-wire 1 meter long and having a resistance of 4.8 ohms. This established a potential drop of 0.0001 volt per centimeter along the slide-wire, and made it convenient to select readily the desired e. m. f. From one end of the slide-wire and from the sliding contact, wires were led either to the string galvanometer or to the filament and grid of the electron

tube; this may be termed the input circuit. In order to simulate as far as possible the electrical conditions which obtain in recording the action currents of living tisues, a resistance representing the resistance of the tissue was introduced in the input circuit in the path from the sliding contact to the galvanometer or the grid of the electron tube, as the case might be. In the great majority of experiments this resistance, termed R_n, was 10,000 ohms, this value being selected as a convenient one which is about the lower limit of resistances found in experiments on nerve, and about the upper limit in the case of muscle preparations. The key which was used to make and break the current was placed in series with the Edison cell and the resistance R_1, the

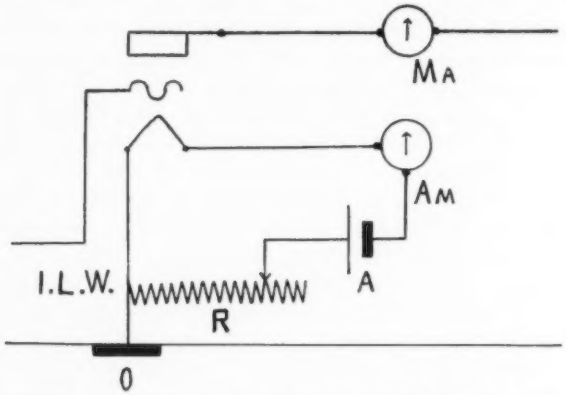


Fig. 9. Wiring diagram of electron tube panel. *I L W*, input lead wires. *O*, brass bar at point of zero potential. *A*, filament-heating battery. *R*, rheostat for controlling filament current. *AM*, ammeter in filament circuit. *MA*, milliammeter for plate current.

input circuit from slide-wire to galvanometer (or to electron tube) remaining closed. The circuital diagram of the standard wiring arrangement reduced to its simplest terms, is shown in figure 8 (cf. 4, fig. 1). This arrangement was the standard with which all the various electron tube methods were compared.

The socket provided for the tubes was mounted on a board, together with the rheostat for controlling the filament current, an ammeter for measuring the same, and a milliammeter for measuring the plate current. The board was permanently wired in accordance with the diagram in figure 9. To a brass bar mounted on the board were soldered

wires, leading to the filament and its rheostat, to the negative pole of the B-battery (plate circuit), and another wire for connection with the source of current to be amplified. This last and a wire soldered to the grid terminal of the socket, may be termed the input lead wires. The brass bar, being at the junctional point of the various circuits, a point normally put to earth and thus maintained at zero potential, may be termed the zero point.

First a series of measurements was made to determine the characteristics of the tubes; that is, to determine the plate current values obtained with various values of filament current (determining cathode temperature), plate potential and grid potential. Doctor Arnold had advised us that the D-tube should be operated at a plate potential in the neighborhood of 200 volts. With this voltage we found that the plate current increased rapidly with the filament temperature till the filament-heating current attained a value of about 1.10 amp.; beyond this point the increase was much less rapid. Van der Bijl (1, p. 175) shows that the device should operate with a filament temperature sufficient to maintain more available electrons than the space charge will permit to travel to the plate; that is, in the present case, with a filament current of 1.10 amp. or more. Most of our experiments were performed with a filament current of 1.20 amp. Figure 10 shows the characteristics of one of the D-tubes[3] with the filament current maintained at 1.20 amp. In figure 10 A the values of plate current, i_b, are plotted against plate voltage, E_b, for different values of grid potential, E_c. In figure 10 B, the plate current is plotted against grid potential at different plate voltages. It should be noted that very different scales are used for grid and plate potentials. Were the curves plotted on the same scale, those in figure 10 B would be much steeper than those in figure 10 A. To make the grid potential zero, (with respect to filament) we connected the grid with the filament through a 10,000 ohm resistance. For various positive and negative values of grid potential we connected the grid with the filament through one or more dry cells in series. From the shape of the curves in figure 10 A it will be seen that the resistance of the tube does not remain constant when filament temperature and grid potential are kept constant, but decreases as the plate voltage is increased; it is a variable dependent on the values of all three determining factors, filament temperature, plate and grid potentials. This is a fact of fundamental importance in dealing with the electron tube, as will be seen later.

[3] The two D-tubes agreed closely in their characteristics.

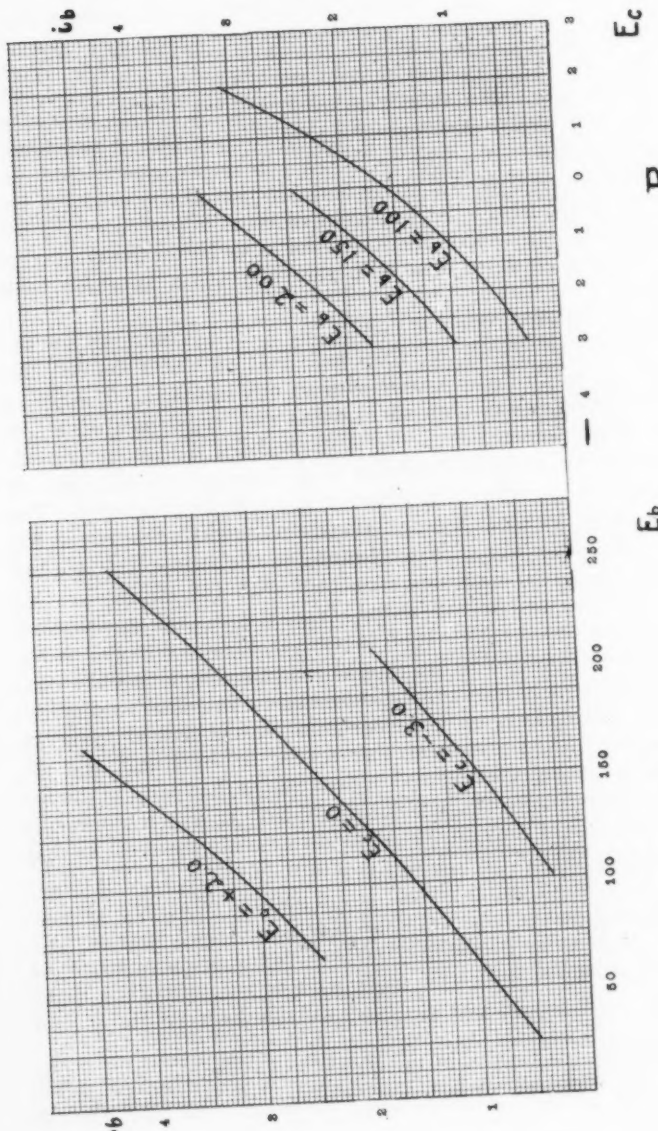


Fig. 10. Characteristic curves of D-tube with filament current of 1.20 amp. (see text). Ordinates, milliamperes. Abscissae, volts.

THE CONDENSER METHOD

The first method tried was the condenser method suggested by Cabot (fig. 5). This method showed promising results at the outset. Both D-tube and L-tube were tried, and the D-tube showed in the preliminary test so much greater amplification that it was thereafter adopted and used regularly in all subsequent experiments. It is possible that more careful study and selection of the values of the various constants would have resulted in some improvement of the performance of the L-tube, but it seemed to us better to concentrate on the analysis of the results with the tube which appeared most promising.

The board with the tube and meters, the A- and B-batteries and the condenser were all placed on tables near that on which the cell and resistances shown in figure 8 were permanently installed. The wiring of these resistances was then so modified and supplemented as to make the entire arrangement conform to the basic scheme in figure 5. The detail shown in figure 9 applies to all experiments, but most of it is omitted in the other diagrams for the sake of simplicity. The protective shunt across the string, already referred to, was connected with those terminals of the switch leading to the string, which, when the switch was open, were not connected with the string. In this way the condenser obtained its initial charge through the shunt before this switch was closed. This is an important safeguard to the string. Figure 11 shows contrasted the standard arrangement and the condenser arrangement of the electron tube circuits intended to simulate those of physiological work. The same e. m. f. was tapped from the resistance slide-wire, representing that arising in the tissue, and the same resistance, R_n, was introduced in each case at that point in the circuit where the resistance of the tissue would be. In some experiments the initial potential of the grid was allowed to remain the same as that of the filament; in that case no cell was introduced at E_c. In others the grid was given an initial "bias," i.e., a positive or negative potential, by inserting one or more dry cells in series between the slide-wire and grid. After a few preliminary tests with the artificial source of current some practical tests were made with action currents in nerve and muscle. The description of these will be reserved for a later section.

After adjusting the tension of the string a photographic record was made with the standard arrangement, showing the excursion due to

the passage of a current through the galvanometer, the circuit being opened and closed by hand with a key (X in fig. 11). Sometimes a metal spring key was used, sometimes a key in which a sharp amalgamated copper point made contact with a cup of mercury. After making this control record we rewired the apparatus for the electron tube test without disturbing the adjustment of the string tension. With the string circuit still open and the shunt closed, we turned on the filament current and established the plate current through R_2. When all adjustments were made we closed the switch leading to the string, and finally opened the protective shunt. The string showed a marked displacement, in some instances disappearing from the field. This indicated a slight leakage of current through the condenser. Measurements showed that when 15 microfarads were used the resistance of

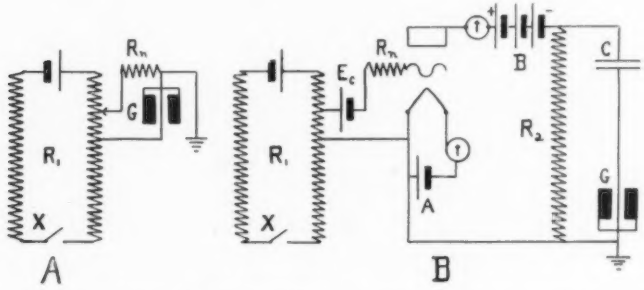


Fig. 11. Comparison of arrangements for experiments on amplification. A, standard arrangement. B, electron tube arrangement, condenser method. Notation as in other figures.

this leak was 32 megohms. It could be avoided by using carefully made mica condensers. A much cheaper way out of the difficulty will be described later. But in most of the experiments we adopted the crude but simple expedient of moving the fibre case of the galvanometer laterally till the string, though deflected by the current, was again in the middle of the field. The input e. m. f. was then applied by making and breaking the circuit at X and the resulting excursions observed and recorded photographically.

It was at this point that we encountered the difficulty already mentioned in recording with the Sandstrom kymograph camera. As soon as the kymograph motor was started the string began to oscillate violently. The same occurred when we turned on the electromagnetic

tuning fork which shows hundredths of a second by its shadow on the film. The Sandstrom kymograph is run by a 110-volt D. C. motor which works on the regular power line; it has a centrifugal governor which maintains a constant speed by breaking the circuit through a part of the armature windings when the speed exceeds this constant value. The action of this governor is attended by visible sparking at the centrifugal contact. The electromagnetic tuning fork was operated on a split circuit from the power mains with a lamp in series and a resistance in parallel. The tuning fork is kept vibrating by means of an electromagnet, on the principle of an ordinary buzzer.

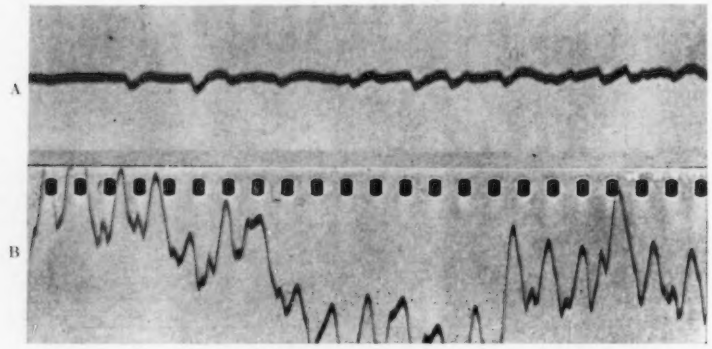


Fig. 12. Oscillations induced by 110-volt kymograph motor in string galvanometer connected with electron tube as in figure 11 B. I_A, 1.10; E, 280; E_c, -1.5; R, 60,000. A, when motor is first started. B, after centrifugal governor has begun to operate (see text). String B. Tension, 1 cm. excursion = 2.75×10^{-7} amp. Speed of film, 15.6 cm. per second. In this and all other photographic records the magnification is 300 diameters. In all records made with electron tubes the following symbols are used:—I_A, filament current; E, plate battery voltage; E_b, plate potential; E_c, initial grid potential; R, by-pass resistance (hitherto designated R_2). In all records made with condenser method condenser capacity was 15 mf.

Both the kymograph and the tuning fork had two features in common, connection with the 110-volt power circuit, and a circuit-breaking contact at which sparking occurred.

In the case of the kymograph motor, some oscillations were visible as soon as the motor was turned on, but they enormously increased in amplitude as soon as the centrifugal governor began to operate with its attendant sparking. (See fig. 12.) It was clearly impossible to

record any experiment with a camera depending on this kymograph motor, at least without elaborate shielding. Such shielding as was mechanically feasible failed to stop the oscillations. We therefore substituted for it the new camera described above, which had just been completed for general use with the string galvanometer. It was then found that the friction of the leather rim of the wheel with the iron disc in the friction drive produced static electricity to such an extent that sparks jumped occasionally from the metal part of the wheel to the disc. The running of this motor caused no oscillation of the string when connected with the electron tube arrangement, except when one of these sparks was seen to jump; then the string made a quick excursion. This difficulty was remedied by grounding both the disc and the wheel of the friction drive, using a copper brush to make continuous contact with the revolving disc. After this change it was found possible to obtain perfectly smooth records with this camera, there being no oscillations in the string induced by the running of the motor. It should be noted that this was a 12-volt motor, run from a storage battery in the room, having no connection with the power mains, and that the motor was completely encased in metal.

The oscillations induced by the tuning fork were found to be considerably reduced in amplitude when, later on, the electron tube and accessories were permanently installed on a lower shelf on the same table with the resistance and switches connected with the galvanometer. The oscillations were still further reduced somewhat when the tuning fork was operated by a 6-volt battery instead of being connected with the power mains (fig. 13). The persistence of oscillations even under these conditions showed the powerful effect of sparking in inducing such disturbances.

The cause of the disturbance was evidently some form of electromagnetic induction, probably due to high frequency currents resulting from sparking, which by their action on the grid circuit, caused disturbances in the plate circuit, by virtue of the rectifying action of the tube. It was feared that a similar disturbance would result from the use of break shocks of an ordinary induction coil for stimulating the tissues to be studied, for the break of the primary current is necessarily attended by a spark. An inductorium was set up in the position commonly used for stimulating, and as large a primary current as is ever used with this coil was broken while the galvanometer was connected with the electron tube as indicated. No excursion of the string resulted unless the hand of the experimenter touched the

primary circuit. With such contact established, the making and breaking of the primary circuit caused excursions of the string. Evidently such sparking as occurred in the inductorium as used in the laboratory, did not suffice to produce any excursions of the string which might confuse an experiment. But it was necessary to insure insulation of the primary circuit from the experimenter, to avoid confusing excursions of the string.

In subsequent experiments the tuning fork was not used, and the constancy of the speed of the motor was relied upon for measurements

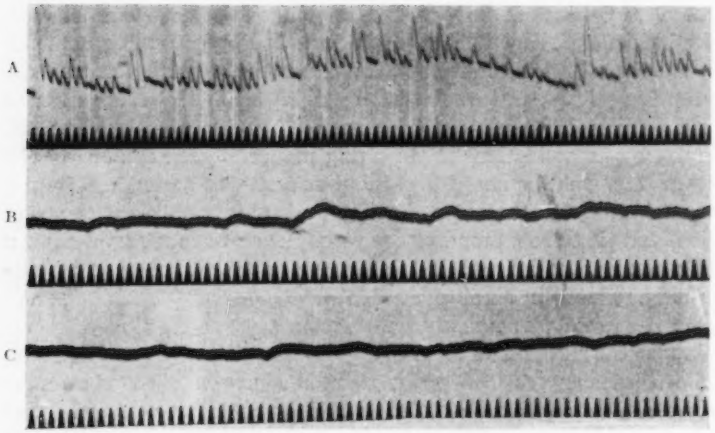


Fig. 13. Oscillations induced by electromagnetic tuning fork. A, string H; tension, 1 cm. = 5.9×10^{-7}. I_A, 1.10; E, 300; E_c, $-$ 1.5; R, 50,000. B and C string B; tension, 1 cm. = 2.75×10^{-7} amp. (as in fig. 12). I_A, 1.10; E, 280; E_c, $-$ 1.5; R, 60,000. In A and B the tuning fork was operated on the 110-volt power circuit; in C it was operated by a 6-volt battery. Time indicated by shadow of tuning fork; each complete vibration = 0.01 second.

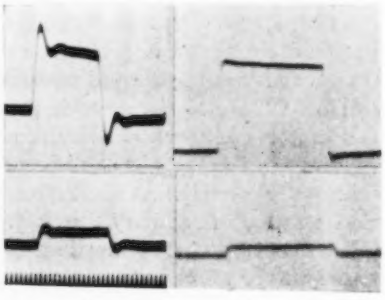
of time. This constancy was found experimentally to be quite adequate for our purposes. A short strip of film with the tuning fork operating was exposed before the experiment, and another one afterwards, by way of speed control.

Referring to figures 5 and 11 B, it will be noted that part of the voltage of the B-battery is used up in the resistance R_2, leaving so much less for the tube. In other words, the plate-to-filament resistance of the tube being in series with the resistance R_2, part of the potential

drop in the circuit occurs in the latter, and only the remainder within the tube. On this account it is necessary to provide a battery of higher voltage than that which would be required to operate the tube without such a resistance in series. It is also necessary to determine, among other things, the value of R_2 which will give the best results.

The question of values of the various resistances and voltages involved will be discussed later in detail. For the present it will suffice to say that the condenser method was tried with various values of plate battery voltage from 113 to 400, and with values of R_2 varying from 10,000 ohms to 120,000 ohms. The results of fairly typical experiments are shown in figure 14. A shows the amplification with the low resistance string; B shows the amplification under comparable conditions with the high resistance string.

Marked amplification in the excursions of the string is obvious in both cases. It is also noticeable that with the electron tube arrangement the decline of the current through the string due to the gradual discharge of the condenser is very small in as brief a time as that shown in the figure; in the case of figure 10 B, amounting to about 0.08 second. From this it follows that for disturbances as brief as the action currents of nerve and skeletal muscle, the distortion from this cause is negligible, as was predicted. It is also noticeable that the amplification is considerably greater in the case of the high resistance string than in that of the low resistance string. This is due to the fact that a low resistance string is relatively



A B

Fig. 14. Amplification by electron tube, condenser method. Upper row, electron tube arrangement; lower row, standard arrangement. In all records of test current with electron tube ΔE_c is change in grid potential used to make record. Other notation as explained in legend to figure 12.

A, string *B;* tension, 1 cm. $= 2.75 \times 10^{-7}$ amp. Upper row: $-I_A$, 1.25; E 240; E_b, 170; E_c, $-$ 3.0; R, 90,000; ΔE_c, 0.0006; R_n, 10,000. Lower row (standard) $\frac{0.0006}{S + 10{,}000}$ (i.e., 10,000 ohms in series with string). Tuning fork shows time in 0.01 sec. intervals.

B, string *G;* tension, 1 cm. $= 5.0 \times 10^{-7}$. Upper row: $-I_A$, 1.25; E, 238; E_b, 170 (approx.); E_c, $-$ 3.0; R, 90,000; ΔE_c, 0.0020; R_n, 10,000. Lower row, $\frac{0.0020}{S + 10{,}000}$. Speed of film, 20 cm. per second.

placed at a disadvantage as compared with a high resistance string when connected in series with the resistances involved in the electron tube circuit, which are very much greater than the resistance of either string. A large external resistance makes less difference to the resulting current if the string resistance is large than if it is small.

We found that when the initial grid potential was zero,—that is, when no cell was introduced to give it a negative bias, the grid circuit absorbed a certain amount of current. This was shown by the fact that the excursions were made considerably smaller when a 10,000 ohm resistance, R_n, was introduced into the grid circuit, than when this resistance was left out. In one experiment this reduction amounted to 32 per cent. When one or more dry cells were inserted in the grid circuit, thus making the grid potential negative to the extent of 1.5 volts or more,[4] the grid circuit took no current. This was shown by the fact that the insertion of as much as 130,000 ohms made no difference whatever in the magnitude of excursion. In this sense the apparatus assumed the function of an electrometer. The consequence is that the electron tube will amplify the electrical disturbance of a tissue of high resistance, very much more than it will that of a tissue of low resistance and the relative gain is greater for a low resistance string than it is for a high resistance string. For instance, in the case of our low resistance string, B, with only 910 ohms resistance, there is no amplification at all in measuring an electrical disturbance set up in a circuit of negligibly small resistance; there is on the contrary a reduction. In other words, when a given difference of potential is applied to the terminals, this low resistance string will give a larger excursion than it will if connected with the electron tube when the same difference of potential is applied to the grid circuit. The conclusion to be drawn from this is that in studying a tissue of low resistance like skeletal muscle, with a low resistance string little or nothing would be gained by use of the electron tube, but that in studying a high resistance tissue such as a nerve trunk very great amplification is to be obtained. This point will be taken up quantitatively later on.

THE TRANSFORMER METHOD

In testing the transformer method it was still necessary to protect the string by placing a condenser in series with it, since the transformer at our disposal was an autotransformer, that is, the windings

[4] A third D-tube used later in some of the final experiments required 3 volts negative grid potential to bring about this result.

were all connected in series, with a number of taps available for connecting the circuits. Thus it was impossible to insulate the secondary circuit from the primary, and consequently without a condenser the string would have been subjected to a large direct current from the B-battery. On the advice of Mr. Colpitts, the coil was connected as a step-down transformer; that is, all four windings were included in the primary, or plate circuit, and only one of the two larger windings was included in the secondary or galvanometer circuit. The reverse arrangement was also tried.

We used values of filament current and plate potential which had given good amplification with the condenser method. It was evident at once that the transformer method was inferior in every respect. Figure 15 shows a comparison of the galvanometric excursions obtained with the standard arrangement, with the condenser method and with the transformer method. In this experiment the essential electron tube values were the same with the transformer method as with the condenser. It should be noted in this connection that the entire plate battery voltage is applied to the plate, none of it being expended in the transformer, whose resistance is negligible compared with that of the tube. The record shows that not only does the transformer method record only the rate of change of the electrical disturbance, but that the amplification of the initial excursion is very much less than that obtained with the condenser method. Reversing the transformer produced a record practically identical with that shown in the figure, but a trifle smaller still. No further experiments were tried with this method.

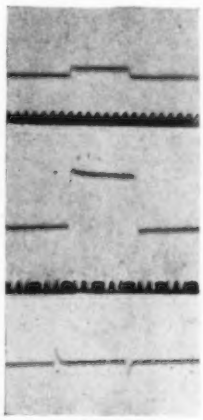


Fig. 15. Comparison of transformer and condenser methods. String *G*.

A, control observation with standard arrangement. Tension, 1 cm. = 6.0×10^{-7} amp. $\frac{0.0020}{S + 10{,}000}$.

B, condenser method. Tension, 1 cm. = 6.0×10^{-7} amp. I_A, 1.25; E, 306; E_b, 163; E_c, 0; R, 50,000; ΔE_c, 0.0020; R_n, 10,000. Tuning fork struck by hand just before observation.

C, transformer method. Tension, 1 cm. = 5.0×10^{-7} amp. I_A, 1.25; E, (same as E_b) 163; E_c, 0; ΔE_c, 0.0020; R_n, 10,000.

THE BRIDGE METHOD

In one experiment we tried the bridge method suggested by Dean (see fig. 4). It proved a difficult matter to balance the four resistances

with sufficient accuracy to keep the string of the galvanometer in the field. And after a balance had been found with difficulty, it was also difficult to maintain it, for the string kept travelling slowly sidewise as a result either of the running down of the A-battery or the B-battery, or of the change of temperature in the tube.

A few records were taken with this method, and as was expected, they showed no great difference from those taken with the condenser method. The chief difference is that there is no falling off during the record of a continued current, such as occurs in the condenser method. On the other hand the gradual shift of base line due to the disturbance of balance mentioned above, though not as rapid as that due to the discharge of the condenser in the other method, is more troublesome in practice. The amplification was also appreciably less with this method than with the condenser. This is probably due to the fact that the experiment was made with the low resistance string whose resistance was so low that it approximated a short-circuit of the bridge. Figure 16 shows records made with the condenser method and the bridge method under approximately comparable conditions, excepting speed of film.

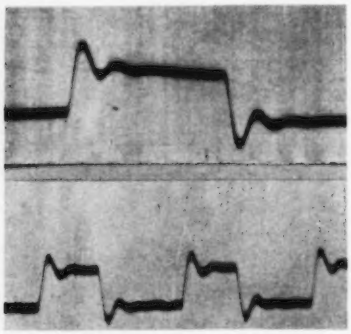


Fig. 16. Comparison of bridge and condenser methods, showing in bridge method absence of decline resulting in condenser method from discharge. String *B*.

A, condenser method. Tension. 1 cm. = 5.50×10^{-7} amp. I_A, 1.25; E, 250; E_c, 0; R, 50,000; ΔE_c, 0.0010; R_n, 10,000. Speed of film, 19.5 cm. per second.

B, bridge method. Tension, 1 cm. = 2.75×10^{-7} amp. I_A, 1.18; E, 223; R_1, 40,000; R_2, 30,000; R_3, 70,700; E_c, 0; ΔE_c, 0.0010; R_n, 10,000. Speed of film 10.7 cm. per second.

These experiments led us to the conclusion that the condenser method was by far the most satisfactory. We proceeded to install a permanent wiring plan whereby we could rapidly shift from the standard arrangement to the electron tube arrangement, wired in accordance with the condenser method, by merely throwing a few switches. This served a twofold purpose. It enabled us to make rapid comparisons between the excursions obtained with the standard arrangement and those obtained with the electron tube, thereby reducing to a minimum the error introduced by change

of tension of the string. It also rendered the method permanently available at a moment's notice for the amplification of any action currents found, in the course of a physiological experiment, to be too small to record with the string galvanometer unaided. This wiring plan is shown in figure 17. In the compactness obtained by installing

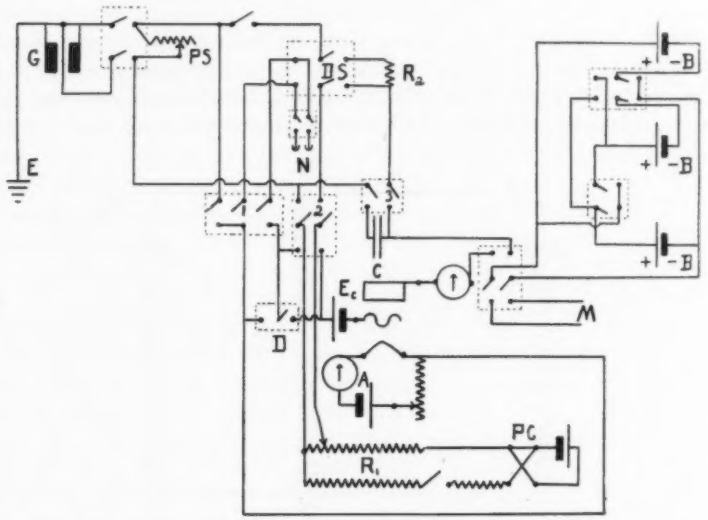


Fig. 17. Complete wiring diagram showing switches for rapid shift from connection of galvanometer directly with tissue or artificial source of current to connection with electron tube. *G*, galvanometer (core grounded). *E*, connection to earth. *PS*, protective shunt. *DS*, switch for connecting galvanometer with tissue or substitution resistance in standard arrangement; in electron tube arrangement this switch is always thrown to right. R_2, in standard arrangement, substitution resistance; in electron tube arrangement, by-pass resistance. *N*, non-polarizable electrodes for connection with tissue. *C*, condenser in series with string. *A*, filament-heating battery. *B*, plate battery in three sections connected with switches for charging them in parallel and discharging them in series. *M*, wires for connection with power mains. E_c, grid bias battery. *PC*, pole-changing switch in source of current used for test or compensation. *D*, single-pole double-throw switch for use in electron tube arrangement. When artificial current is used for test or calibration this switch is thrown to left to short-circuit tissue leads; when recording action currents it is thrown to right and switch *2* is open, thereby disconnecting test current circuit from grid circuit.

In standard arrangement switches *1* and *3* are open, switch *2* is closed upwards. In electron tube arrangement *1* and *3* are closed, *DS* is thrown to right, *2* is closed downward (or open if *D* is to right).

Cf. reference 4, fig. 1.

all the apparatus on shelves of the same table which supported the resistances and switchboard of the old equipment we found the double advantage of convenience and of minimizing the disturbances due to induction in the grid circuit.

THE SEARCH FOR MAXIMUM AMPLIFICATION

General considerations. Having chosen the condenser method as indicated in figure 5 as that which was most worth developing, it remained to determine if possible how to find those values of the four variables, filament current, grid potential, plate potential, and by-pass

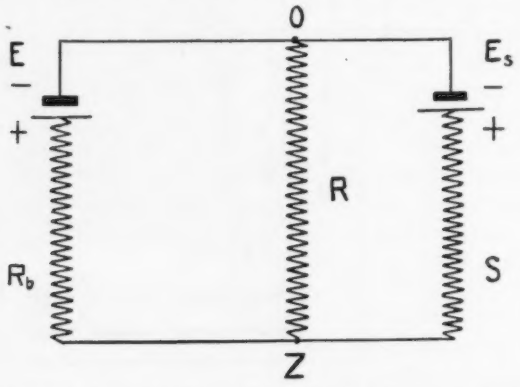


Fig. 18. Simplified schema for analysis of condenser method (see text). E, plate battery; R_b, plate-to-filament resistance of tube; R, by-pass resistance (designated R_2 in figs. 5 and 11); E_s, battery susbtituted for condenser; S, string of galvanometer; O, junctional point assumed for convenience to be at zero potential in analysis; Z, junctional point actually put to earth, here assumed to be point of variable potential.

resistance, R_2, which would yield the maximum possible amplification. Our aim was to find the maximum current in the string which could be made to result from a given change in the difference of potential between grid and filament.

For purposes of analysis we may look on the system as simplified in accordance with the schema shown in figure 18. In this the electron tube and the string are shown simply as resistances, and the condenser is replaced by a battery. In reality a difference of potential is maintained between the plates of the condenser, equal during the resting state, to the voltage drop in R (representing R_2 in fig. 11), and the

capacity of the condenser is so large that for such brief times as we are dealing with, this difference of potential remains practically constant; for purposes of argument we may look on the capacity as infinite. It acts like a battery whose voltage exactly equals the fall of potential in R in the resting state, and it is therefore so represented in the analysis.

In practice the zero point which is grounded, and therefore at zero potential, is that point in the system which is connected to the string, the positive end of R, the filament and one side of the input circuit. But in the following discussion it will simplify matters to assume that the point of zero potential is that which is connected with the negative terminal of the B-battery, the negative end of R and the negative plate of the condenser (O in fig. 18). The potentials of all other points in the system are positive with respect to this point, and we may assign to them absolute instead of relative values if we consider the potential of this point fixed at zero. When the difference of potential between grid and filament is raised the resistance of the tube is decreased, and the portion of the total plate battery voltage used up in the tube is decreased. Consequently a rise in potential occurs at that point in the system at which the filament, the positive end of R, and the string are connected together (Z in fig. 18). Since the negative plate of the condenser is taken to be fixed at zero potential, the positive plate, (in the present schema, the positive terminal of the battery E_s) may be taken to be at a fixed potential E_s. When the potential of the point Z is raised, a current will flow through the string from this point to the positive condenser plate. If the potential difference in the grid circuit is lowered the reverse process occurs, everything happening in like manner but in the opposite sense; but to avoid confusion of terms it will be simplest to consider in every case the current resulting from raising the grid potential.

The quantities we must deal with may be designated as follows:

E = total plate battery voltage.
E_b = difference of potential between plate and filament.
E_c = difference of potential between grid and filament, grid potential.
E_s = difference of potential between plates of condenser.
R_b = plate-to-filament resistance of tube.
R = by-pass resistance. (Designated as R_2 in fig. 11.)
S = resistance of string in galvanometer.
i_b = plate-to-filament current within tube.
i_r = current flowing in R.
i_s = current in string galvanometer.

In the resting state a constant current flows through the tube and the resistance, R, in series, while no current flows in the string; in other words $i_b = i_r$, and $i_s = 0$. It is our aim to make the current in the string, resulting from a given change in grid potential as great as possible; in other words, to make $\frac{di_s}{dE_c}$ a maximum.[5]

The immediate cause of the string current, i_s, is the rise of potential at the point Z, or the difference of potential between this point and the positive plate of the condenser; this results directly from the diminution of voltage drop within the tube, ΔE_b. This in turn results directly from the change in resistance of the tube. It is therefore our aim to make the change in tube resistance as great as possible without introducing factors which will offset the advantage thus gained. With these considerations in mind we may attempt to find in the tube characteristics a clew to the best method of fulfilling the desired conditions. The problem resolves itself into two phases; (a) how to make a given change of grid potential produce the greatest proportional change in tube resistance; (b) how to make a given change of tube resistance produce the greatest current in the string. We shall consider (b) first, since the result of this consideration will influence our mode of attack upon (a).

Reduced to its simplest terms the fall of potential at the point Z is that which occurs at the junctional point of two resistances connected in series with a battery of fixed voltage, when one resistance changes and the other does not. This may be illustrated simply in figure 19 (R_b and R being transposed for convenience). Let the negative pole of the battery be kept at zero potential, and the positive pole at the potential E. Then if R remains fixed, the potential of the junctional point, E_b, will change when the resistance R_b changes. It can be shown that for any given ratio between R and the initial value of R_b, the change in E_b, (dE_b) depends directly on the *proportional* or percentage change of R_b; i.e., on $\frac{dR_b}{R_b}$ or $d \log R_b$. It can also be shown that for a fixed value of E the change in E_b for a given proportional change in R_b is greatest when R is made equal to R_b. In other words

[5] For those unfamiliar with this notation it may be explained that Δ signifies a definite increment (or decrement if negative) of the quantity designated; d signifies an infinitesimal increment; $\frac{dy}{dx}$ signifies the rate at which a variable y is changing per unit change of a related variable x at any given time.

$\frac{dE_b}{d \log R_b}$ attains its maximum value $\frac{E}{4}$ when $R = R_b$. When this condition obtains, since E_b is half of E, the value $\frac{E}{4}$ will equal $\frac{E_b}{2}$.

If we are free to make the battery voltage, E, as large as we like, and to make R correspondingly large in order that the actual plate voltage in the tube (E_b) may be kept within proper limits, we can increase the value of $\frac{dE_b}{d \log R_b}$ (change of potential at Z for a given proportional change in R_b) still more than is possible if E is fixed and R is made equal to the initial value of R_b, as was assumed above.

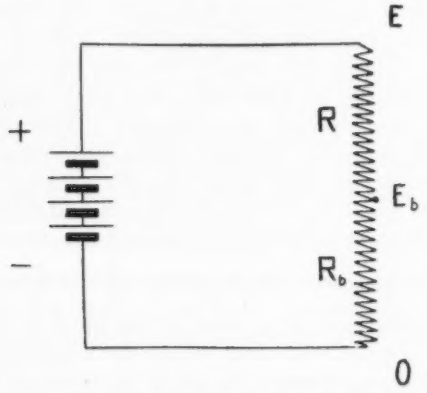


Fig. 19. Schema to illustrate change of potential at junction of resistance in series (see text).

When E and R are thus increased, $\frac{dE_b}{d \log R_b}$ approaches the value E_b as E becomes infinitely large. In other words if we operate the tube at its proper plate voltage we can make the desired change of potential almost twice as great by making the battery voltage, E, very much larger than the plate voltage E_b, as by making it only twice as large as E_b. On this account it would appear desirable to make the battery voltage as large as possible and to increase R correspondingly. In practice the difficulty and danger of having in the laboratory a battery of much more than twice the 200 volts at which the plate of one of these tubes should operate, would be so objectionable as to offset any such small increase in potential as could be had in this way.

From the above we have seen that for our purpose we should seek the maximum proportional change of resistance in the tube for a given change of grid potential. This leads us to study the tube characteristics. Van der Bijl (1, p. 180) gives us the general equation correlating plate-to-filament current with plate and grid potentials, $i_b = \alpha(\gamma E_b + E_c + \epsilon)^2$, where α, γ and ϵ are constants depending on the structure and properties of the tube, and the other symbols have the meanings already assigned to them. Langmuir (5) has given a similar formula chiefly differing from van der Bijl's in that the exponent is 3/2 instead of 2. It is generally held that some portions of the characteristic curve obey more nearly the formula of van der Bijl, and other portions the formula of Langmuir. The relations embodied in these formulae are expressed graphically in figure 10. Referring to figure 10 B, showing the relation between plate current and grid potential, and bearing in mind that at a given plate voltage plate current varies inversely with the resistance of the tube, we see that the porportional change of resistance for a given change of grid potential becomes greater and greater as the grid potential becomes more and more negative. It can be shown mathematically that if either van der Bijl's or Langmuir's equation holds, the maximum value of $\frac{d \log R_b}{dE_c}$ (i. e., proportional change of tube resistance for a given change of grid potential) is obtained at the point in the curve where the current i_b becomes zero: i.e., where the resistance becomes infinite. If we go too far toward this point on the curve we shall defeat our ultimate aim, for the current in the string i_s is always less than i_b, and if this is made excessively small a corresponding limit is placed on i_s. We must seek that point in the characteristic curve of the tube as determined by cathode temperature and by E_b and E_c which, everything considered, will give us the greatest current in the string for a given change in E_c.

Two ways are open to seek this end, complete mathematical analysis based on the formulae expressing the tube characteristics, and actual experiment with a large variety of systematically chosen values of the independent variables,—filament current, battery voltage, grid potential, and by-pass resistance. Both methods were tried, and the results will be described in turn.

Before leaving the general features of the problem it should be noted that with a given grid potential, E_c, the current increases more rapidly as the plate voltage, E_b, is increased than it would if the tube

resistance were fixed. That is, the increase in plate voltage actually lowers the resistance of the tube. The effect of this is to reduce the amplification which would otherwise be obtained. The reason is as follows: Taking our standard case in which the grid potential is raised by a small amount, this change, ΔE_c, causes a diminution of the tube resistance, R_b. But since the plate voltage is not fixed, but depends on the ratio between R_b and R in accordance with the relation $\frac{E_b}{E} = \frac{R_b}{R_b + R}$, the diminution of R_b serves to lower E_b. This in turn, because of the above noted characteristic of the tube, increases the tube resistance. Thus the change in resistance which a change in E_c tends to produce is in part wiped out by the resultant change in the opposite sense caused by the resulting change in E_b.

Mathematical analysis. In this section we are indebted to Dr. E. L. Chaffee for developing a method of analysis and for many helpful criticisms and suggestions.

We shall continue to assume the condenser replaced by a battery and the point *0* (fig. 18) to be maintained at zero potential. The obvious method of searching for the law of maximum amplification would be to find an expression for $\frac{di_s}{dE_c}$ in terms of all the variables involved (except filament current which we may regard in the mathematical treatment as fixed), and then by differentiating for each variable in turn to find at last the value for each which will give the absolute maximum.

Following this method we may begin with the formulae

$$i_s = i_b - i_r \text{ and } i_r = \frac{E_s + Si_s}{R}$$

derived from Kirchhoff's laws. Combining these with van der Bijl's law for plate current, $i_b = \alpha(\gamma E_b + E_c)^2$, (neglecting the quantity ϵ which is insignificant), we obtain the formula

$$i_s = \alpha(\gamma E_b + E_c)^2 - \frac{E_s + Si_s}{R},$$

from which by a simple process of algebra we obtain the formula

$$i_s = \frac{R\alpha(\gamma E_b + E_c)^2 - E_s}{R + S}.$$

This expresses the current in the string in terms of the controllable quantities. But when we attempt to differentiate this expression with respect to E_c, making allowance for the fact that E_b is a function of E_c, we encounter insoluble expressions, and the method proves impossible.

An ingenious method has been proposed by Doctor Chaffee whereby a very close approximation to the true value of $\frac{di_s}{dE_c}$ can be expressed in comparatively simple terms. The method is as follows:—

Referring to figure 18 and applying Kirchhoff's laws we have the following fundamental equations:

$$E_s + i_s S - i_r R = O, \tag{1}$$

$$i_b = i_r + i_s, \tag{2}$$

$$i_r R = E - E_b. \tag{3}$$

Combining (1) and (2) and multiplying through by R, we have

$$E_s R + i_b S R - i_r R\,(S + R) = O;$$

substituting (3) and rearranging, we have

$$i_b = \frac{(E - E_b)\,(R + S) - E_s R}{RS}. \tag{4}$$

Differentiating,

$$\frac{di_b}{dE_b} = -\frac{R + S}{RS}. \tag{5}$$

Changing (2) to read $i_r = i_b - i_s$, and combining it with (1), we have,

$$E_s + i_s S - i_b R + i_s R = O;$$

from which

$$i_b = \frac{E_s}{R} + \frac{(R + S)}{R}\, i_s \tag{6}$$

Differentiating,

$$\frac{di_b}{di_s} = \frac{R + S}{R}. \tag{7}$$

Thus far the equations are rigorous. At this point we make an assumption which introduces a slight inaccuracy, but greatly simplifies the treatment. We assume that the tube is operated under conditions such that the curve of i_b plotted against E_b is a straight line,

and that the slope of this line is not altered when it is displaced to right or left by a change of E_c. For the small changes of E_b and E_c involved in this work the assumption is a close approximation to the truth. The operation of the system on this assumption is shown graphically in figure 20. Two curves of i_b plotted against E_b for slightly different values of E_c, are shown side by side. The slope of these curves in the region used is such that $\frac{di_b}{dE_b} = \frac{1}{r}$; r, may thus be

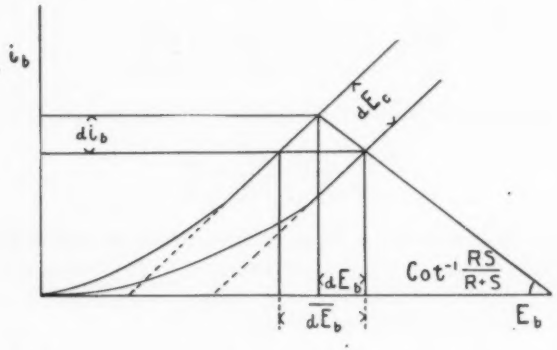


Fig 20. Graphic representation of simplified mathematical analysis by Doctor Chaffee (see text).

regarded as the virtual resistance of the tube. The expression for the curve now becomes

$$i_b = \frac{1}{r}(E_b + \mu(E_c - E_o)). \tag{8}$$

In this expression r, μ, and E_o are constants.

Then $$\left(\frac{di_b}{dE_b}\right)_{E_c} = \frac{1}{r}, \text{ and } \left(\frac{di_b}{dE_c}\right)_{E_b} = \frac{\mu}{r}.$$[6]

We may now seek an expression for $\frac{di_a}{dE_c}$, the desired quantity;

$$\frac{di_a}{dE_c} = \frac{di_a}{di_b} \times \frac{di_b}{dE_c}.$$

[6] The expression $\left(\frac{di_b}{dE_b}\right)_{E_c}$ means $\frac{di_b}{dE_b}$ when E_c is kept constant.

Substituting (7) in the above we have

$$\frac{di_s}{dE_c} = \frac{R}{R+S} \times \frac{di_b}{dE_c}.$$

The next step is to find $\frac{di_b}{dE_c}$ under the conditions in the system.

$$di_b = \frac{\partial i_b}{\partial E_b} \cdot dE_b + \frac{\partial i_b}{\partial E_c} \cdot dE_c.$$

$$\frac{di_b}{dE_c} = \left(\frac{\partial i_b}{\partial E_b}\right)_{E_c} \cdot \frac{dE_b}{dE_c} + \left(\frac{\partial i_b}{\partial E_c}\right)_{E_b}.$$

Substituting the partial derivatives above this becomes:

$$\frac{di_b}{dE_c} = \frac{1}{r} \cdot \frac{dE_b}{dE_c} + \frac{\mu}{r}. \tag{9}$$

Now in this system any change in E_c causes a change in E_b. From the analysis of the system in accordance with Kirchhoff's laws as indicated in equations (1) to (5) any change in E_b must be correlated with i_b in accordance with the formula

$$\frac{di_b}{dE_b} = -\frac{R+S}{RS}. \tag{5}$$

Therefore any change in E_b produced by a change in E_c must cause a corresponding change in i_b which when plotted as in figure 20 will fall on a line whose slope is indicated by $\frac{di_b}{dE_b}$, i.e., the cotangent of whose angle with the horizontal is $-\frac{R+S}{RS}$. Let $\overline{dE_b}$ be the differential of E_b when i_b remains constant, i.e., the change of E_b required to restore i_b to its original value when it has been altered by a change in E_c; $\overline{dE_b}$ will then represent the horizontal distance between the two curves. We shall use dE_b express the actual differential of E_b in the system as resulting from a change of E_c.

By definition

$$\frac{\overline{dE_b}}{dE_c} = \left(\frac{\partial E_b}{\partial E_c}\right)_{i_b} = -\frac{\frac{\partial i_b}{\partial E_c}}{\frac{\partial i_b}{\partial E_b}} = -\frac{\frac{\mu}{r}}{\frac{1}{r}}.$$

Therefore
$$\frac{\overline{dE_b}}{dE_c} = -\mu. \tag{10}$$

By inspection of figure 20 it is evident that

$$\frac{\overline{dE_b} - dE_b}{di_b} = -r. \tag{11}$$

Combining this with (5) we get

$$\frac{\overline{dE_b} - dE_b}{di_b} \cdot \frac{di_b}{dE_b} = \frac{(R+S)r}{RS}.$$

Simplifying this equation and adding 1 to each side,

$$\frac{\overline{dE_b}}{dE_b} = \frac{(R+S)r + RS}{RS}. \tag{12}$$

Combining this with (10) we get

$$\frac{dE_b}{dE_c} = -\frac{\mu RS}{(R+S)r + RS}. \tag{13}$$

Substituting this expression in (9) and reducing we get

$$\frac{di_b}{dE_c} = \frac{\mu(R+S)}{(R+S)r + RS}. \tag{14}$$

Finally by combining this with (7) we obtain the desired formula

$$\frac{di_s}{dE_c} = \frac{\mu R}{(R+S)r + RS}. \tag{15}$$

This equation enables us to calculate the quantity $\frac{di_s}{dE_c}$, upon which amplification depends, in terms of two known resistances and two constants which may be obtained from the characteristic curves of the tube, and these curves may readily be obtained by direct experiment. Evidently to make this expression large we should choose large values of μ and R, and small values of r and S. But whereas the value of the expression is directly proportional to μ, its increase with increase of R is relatively slight.

Experimental. Most of the experiments made in order to determine the method of obtaining the maximum amplification were performed before the mathematical analysis by Doctor Chaffee had been developed. The end result being a function of four independent variables, it was difficult to isolate the effect of varying each quantity by itself. A large number of experiments was necessary. In order to make the basis of comparison uniform we kept the resistance R_n, representing the nerve, constant at 10,000 ohms. It has already been mentioned that with the tubes first used, when an initial negative grid potential amounting to 1.5 volts or more was applied, the resulting excursion of the string became independent of the value of this resistance. Therefore the insertion of R_n under this condition became unnecessary. Early in the experiments we found that considerably greater amplification was obtained with a negative grid potential than without. Thereafter all experiments bearing on maximum amplification were made with at least 1.5 volts negative grid potential. For the same reason of uniformity of comparison it was necessary to use the same string throughout a series of experiments, or at least to keep the string resistance constant. Most of the earlier quantitative comparisons were made with string G, having a resistance of 20,000 ohms. Later this string lost its conductivity, and we were left to complete the experiments with only string B, having a resistance of 910 ohms. Therefore in order to make the experiments quantitatively valid we introduced a resistance of 19,000 ohms in series with string B to make the resistance of that part of the circuit the same as it had been with string G. In a few experiments we placed 20,000 ohms instead of 19,000 ohms in series with string B for convenience. In comparing results, amplification was defined as the ratio between the excursion of the string when connected with the electron tube, caused by placing a given change of potential (one or two millivolts), on the grid, and the excursion obtained by placing the same difference of potential in series with the string and the resistance R_n arbitrarily chosen to represent the resistance of a nerve. Clearly the amplification with a given arrangement depends on the magnitude of resistance selected to represent the nerve, since as already stated this magnitude has no effect on the excursion obtained with the electron tube under the best conditions of operation, whereas it makes a great difference in the excursion obtained without the tube. In giving figures for amplification the assumed value of R_n must be stated.

In order to simplify the problem as far as possible we decided to select a standard value of filament current and to use it in all quantitative experiments in order that this should not enter as a variable into the result. As already stated we found that the plate current increased rapidly with increase of filament current, until the latter reached a value of about 1.1 amperes, and after this, much less rapidly. Van der Bijl has shown (1, p. 175) that a tube should operate in the latter part of its curve; that is, under the condition that the plate current varies little with change of filament current. It is also easier to maintain uniform working conditions with the conductivity of the tube as insensitive as possible to changes of cathode temperature. On the other hand it is desirable not to operate the tube with too high a cathode temperature, since to do so shortens the life of the tube. For these reasons 1.20 amperes was selected as the standard filament current and used in a great majority of the experiments. In a few cases this was varied in order to determine the effect of doing so.

The values E, R and E_c then remained to be studied with a view to choosing the optimum value of each. In our earlier experiments we varied E_c, keeping E and R constant, and then varied R, keeping E and E_c constant. The results were difficult to interpret, since by changing either one of these variables we changed indirectly the value of E_b, the actual voltage drop within the tube, thereby changing the tube resistance and producing a complex instead of a simple change of conditions. For instance, if E_c was changed, keeping E and R constant, the tube resistance was changed, and that portion of the total battery voltage, E, used up in the tube was correspondingly changed. The consequence was that we were not only studying a different part of the i_b, E_c curve but a different part of the i_b, E_b curve as well. In like fashion a change of R was complicated by a similar change of E_b. We finally decided to carry out some experiments keeping E_b constant at approximately 200 volts, the value of the plate potential recommended by Doctor Arnold as giving the best results with this type of tube. In this way we sought to determine under comparable conditions the best values of grid potential and total battery voltage, and as a corollary of the latter the value of the by-pass resistance, R. As nearly as we could judge from an effort to determine the influence of plate potential as distinguished from that of other factors in the earlier experiments, the best amplification was obtained in the neighborhood of 200 volts, although this value was by no means critical: amplification almost as great was obtained with values higher and lower than this.

Adherence to approximately 200 volts as a standard therefore appeared advisable. If the plate potential, E_b, is to be kept constant the problem of choosing the total battery voltage, E, becomes secondary to that of choosing the value of the by-pass resistance, R. That is, the choice of E is the means to the end of obtaining the desired ratio between by-pass resistance, R, and tube resistance, R_b.

Preliminary experiments had shown the greatest amplification to be obtained with values of E_c between -1.5 and -4.0, and with values of the ratio $\frac{R}{R_b}$ lying between 0.4 and 0.9. In order to carry out a complete experiment to show the effect of varying the ratio $\frac{R}{R_b}$ at different values of grid potential, and of varying the grid potential at different values of the resistance ratio, we made a careful series of measurements of the tube resistance with the plate potential kept at 200 volts and the following values of grid potential: -2.0, -2.5, -3.0, -3.4 and -3.9. We selected the following values of the battery voltage, E: 278, 302, 322, 342, 362, as giving with the desired plate potential a series of resistance ratios ranging from 0.4 to 0.9. Each series of observations was to begin with a battery voltage of 278. We therefore calculated from the above measurements of tube resistance the value of by-pass resistance, R, which for each value of grid potential would make the plate potential 200 volts with a battery voltage of 278. In this way each series of observations would start with the same value of E, the same value of E_b, and consequently the same resistance ratio $\frac{R}{R_b}$, namely 0.4. With these calculations made beforehand the experiment was begun, and on the first observation of each series, the plate current was read with the milliammeter. Thereafter each time the battery voltage was increased the resistance, R, was correspondingly increased just enough to maintain the same value of the plate current. In this way we could be certain that we were maintaining the same value of the plate potential, E_b, so long as the grid potential and filament current were not altered, and these were carefully controlled. Having completed such a series with a grid potential of -2.0, we began the second series with a grid potential of -2.5, using the same steps of battery voltage and beginning with the previously calculated value of R, noting the resulting value of the plate current and again keeping this constant throughout the series. In this way five series were carried out, but the last three were incom-

plete, since we had not resistances enough to maintain the higher ratios when the tube resistance became very high as a result of using a large negative grid potential. At frequent intervals during the experiment the apparatus was rearranged according to the standard wiring plan, and control observations were made with the current sent directly through the string. From these observations we plotted a curve showing the gradual change of string tension due to heating of the galvanometer, which enabled us to apply the necessary corrections to make the comparison valid. Owing to slight inaccuracies in the previous measurements of tube resistance and possibly to slight

TABLE 1

E_c	R/R_b				
	0.40	0.52	0.62	0.75	0.88
			A		
−2.0	10.9	11.4	11.2	10.8	10.7
−2.5	10.8	11.0	11.1	10.9	10.5
−3.0	10.9	11.0	11.0	10.6	
−3.4	10.6	10.5	10.5		
−3.9	9.6	10.1			
			B (corrected)		
−2.0	11.7	12.4	12.8	13.0	13.4
−2.5	11.6	12.0	12.7	13.1	13.1
−3.0	11.7	12.0	12.5	12.7	
−3.4	11.4	11.5	12.0		
−3.9	10.3	11.0			

changes in the behavior of the tube, we were unable to keep the resistance ratios precisely the same in each series of observations. But by means of the milliammeter readings of the plate current we were able to be sure that the plate potential remained constant during each series, and we were able to maintain it at nearly the same value through all of the series. The results are tabulated in table 1 A, the figure for amplification being given on the basis of 10,000 ohms as the value of R_n.

From this experiment it would appear that the greatest amplification occurred with a grid potential of about −2.0 volts and with a value of the by-pass resistance about half as great as that of the

tube. On the other hand it is evident from this and from the preliminary experiments that these values are not at all critical; in fact very nearly as great amplification is obtained with values of all the quantities varied over a wide range; in the case of grid potential all the way from -1.5 to -4.2; in the case of by-pass resistance all the way from equality with the tube resistance down to less than half of this value.

After the completion of this experiment the mathematical analysis given above was developed, and the results of experiments were thereupon compared with the calculated values of amplification based on the formula deduced by Doctor Chaffee, $\frac{di_a}{dE_c} = \frac{\mu RS}{(R+S)r + RS}$. In making these calculations the values of μ and r were obtained by making careful measurements of the tube characteristics, plotting curves of these on coördinate paper and measuring the slopes of the curves at the proper points.

The comparison of observed and calculated values showed in general a striking agreement. In those experiments in which no initial negative grid potential was applied and in which therefore the grid circuit absorbed current, there was a wide discrepancy between the calculated and observed values. But inasmuch as the mathematical analysis takes no account of such action in the grid circuit this particular discrepancy is to be expected. It is furthermore unimportant, since the amplification is much less under this condition than with a negative grid potential. Therefore only those results in which there was a negative grid potential, and the system operated as an electrometer, need be considered. In all such cases the greatest discrepancy found amounted to only about 15 per cent; in a majority of observations it was less than 5 per cent; in some observations there was practically none. On the other hand in the experiments just described in which conditions were most carefully controlled with reference to constancy of plate potential, we found an inverse correlation which, though slight, was constant, and therefore led us at first to think that the formula failed to deal adequately with all the factors. According to the formula the 66 per cent increase in by-pass resistance between the second and fifth observations in the top row of table 1 A, in which grid potential was kept at -2.0 volts, should have caused an increase in amplification amounting to 8 per cent. In reality it caused a decrease in amplification amounting to 7 per cent. Similarly in the second series when the grid potential was kept constant at -2.5 volts a 60 per cent increase in

by-pass resistance, instead of causing the calculated 6 per cent increase in amplification, did cause a 4 per cent decrease in amplification. As stated above, in each of these comparisons grid potential remained constant, and plate potential was kept constant with the aid of the milliammeter in the plate circuit. Therefore, since we were working at precisely the same point in the characteristic curve of the tube, the greatest source of inaccuracy, viz., the determination of r and μ depending on the slopes of the characteristic curves, was completely eliminated.

An explanation of the apparent discrepancy was found in the leakage of the condenser. It has already been noted that leakage occurred and that the resistance of fifteen one-microfarad condensers connected in parallel, (the arrangement regularly used) amounted to 32 megohms. When the voltage drop in R is increased, as in this experiment, by increasing the resistance ratio and with it the total battery voltage, the difference of potential between the condenser plates is increased and with it the current in the string due to leakage. This current is great enough to place the string under appreciably increased tension on account of displacement. This increased tension reduces the excursion resulting from a given current, and the greater the displacement the greater will be the reduction. In this way it seemed probable that the increase in amplification which should have resulted from increasing the ratio $\frac{R}{R_b}$, was more than nullified by the concomitant increase in string tension. An experiment was made to test this point. The string was adjusted to the same initial tension that was used in the experiment described, and then deflected by a current of approximately the same magnitude as that resulting from the condenser leakage in the case of the lesser voltage drop. A small difference of potential was then applied and the excursion measured. Then the string was further deflected by a current as great as that leaking through the condenser under the greater voltage, and the excursion resulting from the same difference of potential was measured. The decrease of sensitiveness caused by the smaller displacement amounted to 17 per cent; the decrease caused by the larger displacement, 26 per cent. A correction for this difference applied to the amplification as observed in the experiment under consideration, sufficed to change the apparent loss of amplification to a gain of almost exactly the right amount to correspond with the calculated value. The agreement between observed and calculated values was thus brought well within the limits of observational

error. The experimental verification of Chaffee's formula thus became as complete and satisfactory as the experimental conditions rendered possible.

In table 1 B is shown the result of the experiment as modified by the corrections for change of string tension due to displacement. This shows the amplification which presumably would have occurred in this experiment had there been no leakage in the condenser.

In table 2 are shown a number of calculated and observed values of $\frac{di_s}{dE_c}$ from experiments with different values of μ, r, R and S. The

TABLE 2

μ	r	R	S	$\frac{di_s}{dE_c} \times 10^4$	
				Calculated	Observed (corrected)
35.7	71,500	50,000	910	4.85	4.95
40.0	67,000	50,000	20,000	3.51	4.05
37.0	62,000	50,000	20,000	3.47	3.30
41.4	69,000	50,000	20,000	3.55	3.83
40.0	71,000	100,000	20,000	3.80	3.80
40.0	71,000	50,000	20,000	3.35	3.42
39.0	65,000	50,000	20,000	3.51	3.75
39.0	65,000	100,000	20,000	3.98	4.02
39.0	65,000	120,000	20,000	4.07	4.11
40.5	69,000	60,000	21,000	3.55	3.99
40.5	69,000	100,000	21,000	3.88	4.30
41.7	71,000	72,000	21,000	3.70	3.85
41.7	71,000	114,000	21,000	3.97	4.23

observed values are corrected for string displacement. They illustrate the agreement between prediction and result; and considering the difficulty in obtaining accurate values of μ and r, they are quite satisfactory.

PRACTICAL DEDUCTIONS

Since the formula for amplification $\frac{di_s}{dE_c} = \frac{\mu R}{(R+S)r + RS}$ is experimentally proved valid, it should be a simple matter to lay down practical rules for obtaining maximum amplification. Four distinct quantities are involved and may be dealt with in turn.

If r could be considered by itself we should want to make it as small as possible since it appears only in the denominator; that is, we

should want to choose the steepest part of the curve correlating i_b with E_b (in fig. 10 A). But since μ is determined in part by r we must consider the two together. Clearly μ should be made as large as possible. But μ is defined as equal to $\frac{\left(\frac{di_b}{dE_c}\right)_{E_b}}{\left(\frac{di_b}{dE_b}\right)_{E_c}}$; that is, it is the ratio between the slope of the i_b, E_b curve and the slope of the i_b, E_c curve, or the relative sensitiveness of plate current to changes in grid potential as compared with its sensitiveness to plate potential. In other words it depends not only on the steepness of the i_b, E_c curve but also on the lack of steepness of the i_b, E_b curve. Therefore making r small, while it serves to reduce the denominator of our expression, also reduces the value of μ and therefore at the same time reduces the numerator. As already stated, the experimental evidence shows that the value of plate potential, E_b, is not critical as regards amplification. Very nearly the same results are obtained with values all the way from 150 to 250 volts. Considering both convenience and efficiency, a value between 160 and 200 volts is to be recommended.

With the value of r thus approximately established, μ should be made as large as possible. This means we should seek the steepest part of the i_b, E_b curve. But this leads us into the region where E_c is positive, and it has already been shown that unless E_c is made negative the grid circuit absorbs current, and in that way detracts from the amplification. We should then seek the steepest part of the curve where this will not occur. The results shown in the table appear to substantiate this view, in that the greatest amplification here is obtained with the smallest negative value of grid potential, -2.0 volts. On the other hand, in other experiments with the same tube under otherwise closely similar conditions we have obtained just as great amplification with a grid potential of -3.0 volts; and in some cases almost as great amplification with -4.0 volts grid potential. It is therefore evident that with this tube the variations in μ due to varying grid potential from -1.5 to -4.0 are not large, and do not make much difference in the resulting amplification.

These figures must not be relied on for all tubes of this type. A new D-tube, already referred to in footnote 4, was found to absorb current with a grid potential of -1.5 volts; it was necessary to use as much as 3 volts negative bias to render the excursions independent of

input resistance, R_n, and to bring the tube to its point of maximum amplification. Each tube should be tested in this respect before it is used. In practice the most convenient arrangement is the insertion of either one or two dry cells (according to the requirements of the individual tube) in the input circuit with the negative terminal connected to the grid.

With regard to by-pass resistance, R, it is seen that when allowance is made for the effect of string displacement due to condenser leakage, an increase in amplification is obtained by increasing its value, provided the total battery voltage is increased at the same time enough to keep the actual plate potential the same. However, the increase in amplification after the by-pass resistance has attained a value of 60 per cent of the tube resistance is very small, and a practical limit is placed on its further increase by the undesirability of installing a battery of much more than 300 volts for so slight a gain as is to be had in this way. The correct value may be readily determined after the battery voltage, E, is established and the tube resistance, R_b, has been measured at a plate voltage of about 200 with the negative grid potential found necessary to prevent the grid circuit from taking current. The formula is $R = \frac{R_b(E - E_b)}{E_b}$. Assuming that the tube is to be operated at approximately 200 volts, E_b, the necessary data are provided.

The value of S is of some importance in determining the amplification; larger excursions are obtained for a given impressed e. m. f. with a low resistance string than with a high resistance string. On the other hand, on account of large resistances necessarily involved in this system this advantage is less than it is in the case of direct recording, except when the tissue is one of very high resistance. Furthermore, in the choice of a string the main consideration—lightness and correspondingly small inertia, which enable a string to respond rapidly to currents of brief duration—is just as important when the string is used with the electron tube as without. The string will therefore be selected with reference to its availability for nerve work in general, and may be regarded as a constant in this system, and not as a variable to be selected.

In some experiments the effect of varying the cathode temperature was tested. Although most of the records were made with a filament current of 1.20 amperes, some were made with 1.25 and some with 1.10. Those made with 1.25 amperes showed little difference in amplification

from those with 1.20. Reducing the filament current to 1.10 amperes in the tube first used caused a slight but appreciable reduction in amplification. For example, in one experiment with the following values: filament current, 1.10; E_c, -1.5; E, 270; R, 50,000 ohms; E_b, 185 volts; and a resistance ratio of 0.46, the amplification on the basis of 10,000 ohms as the value of R_n, was 10.3;—the greatest amplification on this basis obtained with any combination (not correcting for string displacement) was 11.5. But with the last tube used reduction of filament current below 1.15 amperes caused a marked loss in amplification. In general it is advisable for the sake of the resulting increase in the length of life of the bulb, to use it at the lowest filament temperature that will give satisfactory amplification.

In choosing the battery voltage, E, to be used when the installation was made permanent, we decided on 135 cells as being exceedingly convenient and at the same time efficient. Forty-five lead storage cells is the maximum number which, connected in series, can be charged efficiently from 110-volt power mains; 45 cells can be conveniently arranged in trays by placing them in nine rows of five cells to a row. Three such trays can be charged in parallel, and when connected in series will give at least 275 volts. As indicated above this voltage is sufficient to operate the tube at very near its maximum efficiency. In practice we have found that our battery when freshly charged has 300 volts on open circuit.

In an earlier section it was pointed out that inasmuch as the electron tube under proper conditions operates as an electrometer, measuring the difference of potential arising in the tissue irrespective of the resistance, the amplification of galvanometric excursion obtained by means of it, depends on the resistance of the tissue, since the excursion obtained with the galvanometer alone depends on tissue resistance as well as on electromotive force. In other words, since a large tissue resistance will reduce the response of the unaided galvanometer to a given e. m. f. but will not reduce the response of the galvanometer when working with an electron tube, a comparison between the two arrangements will show a greater gain in the case of a large resistance than in that of a small one.

This fact is shown quantitatively in figure 21. Here the amplification is plotted for each of the three strings against the value of R_n expressed in thousands of ohms. The curve for string G (resistance 20,000 ohms) is plotted on the basis of an amplification of 11.4 (corrected) at 10,000 ohms tissue resistance, this being a value obtained

in a number of experiments and very nearly the greatest value obtained at all,—in one experiment Chaffee's formula agreed exactly with observation in giving this value. The curves for strings B and H, with resistances of 910 and 12,000 ohms, are based on the calculated amplification under those experimental conditions in which this precise agreement was found; that is, the proper quantity was substituted for 20,000 as the value of S in the formula.

It is notable that as mentioned earlier, amplification is replaced by reduction with the low resistance string when the tissue resistance

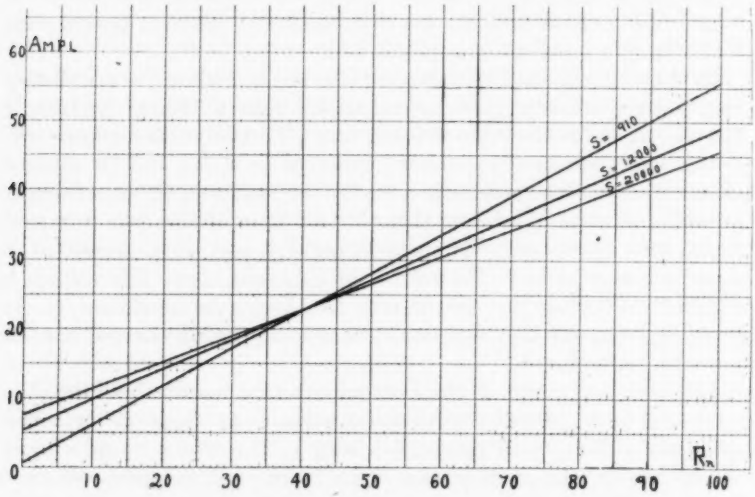


Fig. 21. Amplification (ordinates) plotted against tissue or input resistance, R_n, (abscissae) in thousands of ohms, for the three strings used in experiments, and for the particular case in which $\mu = 40$, $r = 71{,}000$ and $R = 100{,}000$.

becomes very small. With string B amplification ceases when R_n is reduced to about 1000 ohms. On the other hand with a tissue resistance of 42,000 ohms the amplification is identical with all three strings, and with greater tissue resistance the string of lowest resistance amplifies the most. With a tissue resistance of 100,000 ohms we obtain with string B a 55-fold amplification, whereas with the 20,000-ohm-string we obtain 45-fold amplification. These curves are only valid for the particular values of μ, r and R selected in this case. We have found that with a different choice of these values different curves are

obtained, similar in character and again all crossing at a point, but at a different point denoting a different value of R_n. Below this point the amplification is always greatest with the string of highest resistance; above it the inverse relation always holds. The curves are of necessity straight lines. It should be understood in estimating from these curves the relative merits of low and high resistance strings that the gain of amplification is not an absolute quantity, but only relative; it is merely the gain in excursion over what would be obtained in recording under similar conditions without the electron tube.

COMPENSATION FOR CONDENSER LEAKAGE

In an earlier section it was stated that in most of our experiments we met the problem of string displacement due to condenser leakage, by simply moving the fiber case laterally and thus bringing the string

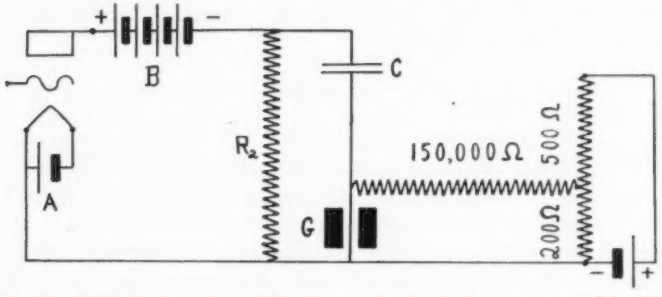


Fig. 22. Method of compensating for condenser leakage, proposed by Doctor Williams.

back into the center of the field. At the suggestion of Dr. H. B. Williams, an electrical method of compensation was tried and found satisfactory. A dry cell was connected with a resistance of 700 ohms. The negative pole of the dry cell was connected with the grounded end of the string. From a point in the resistance separated from this terminal by 200 ohms, connection was made with the other end of the string through the resistance of a graphite line drawn on ground glass, having a resistance of about 150,000 ohms. The arrangement is shown in figure 22. In this way enough current is tapped off from the dry cell circuit to neutralize the current in the string due to condenser leakage, and yet the resistance of the shunt circuit around the string is so much

higher than that of the string itself that it does not act to any considerable extent as a short-circuit. The combination described approximately balanced the condenser leakage, so that when the protective shunt was opened very little displacement occurred. The amplification with this arrangement was measured and compared immediately afterward with the amplification obtained with exactly the same values of the usual constants and with the ordinary method of bringing the string into the field by moving the fibre case. The excursion obtained with electrical compensation was slightly the larger of the two. This shows that if there is any loss of efficiency in this method it is less than the apparent loss of efficiency caused by the increased tension due to the usual displacement. If a standard value of each of the variables is adopted as a matter of permanent installation it is a simple matter also to keep permanently installed a dry cell with the necessary resistances, and to effect electrical compensation by the simple throwing of a single switch. By tapping from the compensating circuit through a still larger resistance, say a megohm, there would be practically no loss of efficiency.

PROPORTIONALITY OF EXCURSION TO INPUT VOLTAGE

For certain purposes it is desirable to know whether our system in amplifying gives a faithful record of the relative values of the disturbances amplified. That is, we should ascertain whether an e. m. f. of 2 millivolts applied to the grid will produce just twice as great an excursion of the string as 1 millivolt. In some experiments this point was put to test. In some cases the excursions resulting from 1 millivolt and 1.5 millivolts were compared in quick succession; in others comparisons were made between 1 and 2 millivolts. The proportionality regularly was good; in most observations it was accurate to within 2 per cent, and in no case was the value out of proportion by more than 4 per cent. This is within the limits of experimental and observational error.

APPLICATION TO PHYSIOLOGY

In order to put the electron tube method to the practical test of recording action currents, experiments were made with the sciatic nerves of both cats and frogs, and with the muscles of the human forearm led off through the skin by the usual method employed in making electromyograms (9).

The experiments on nerves will be described first. The resistance of a given length of a frog's sciatic nerve is very much greater than the resistance of the same length of a cat's sciatic nerve. Therefore the action current in the frog's nerve should be susceptible of far greater amplification than that in the cat's nerve, since the resistance in the tissue serves to reduce the current obtainable in the galvanometer when directly connected with the nerve, whereas we have seen that this resistance causes no reduction in the excursion of the galvanometer when used with the electron tube.

A preliminary experiment was made with a cat's nerve, using the heavy string (string *B*). This was done before the best values of the variables for amplification had been found. The result showed amplification, but not as great as was obtained under the more favorable conditions developed later. After the experiments on maximum amplification had been completed and the permanent installation had been arranged as described above, an experiment was made to illustrate the amplification of the monophasic action current in a frog's nerve; in this experiment the new string, *H*, with a resistance of 12,000 ohms, was used. The sciatic nerve of a good-sized frog was placed in a moist chamber with stimulating electrodes on the central end, and the distal end laid across non-polarizable boot electrodes 15 or 20 mm. apart. The nerve was crushed at a point midway between the boot electrodes to render the response monophasic. The resistance of the section of nerve and the electrodes in circuit with the string was measured by substitution, and found to be 85,000 ohms at the beginning of the experiment, rising as a result of drying to over 100,000 ohms at the end. Such a high resistance as this should prove favorable to great amplification, as indicated above. Specifically, with the combination of tube constants employed, which had previously shown 10.4-fold amplification with 10,000 ohms for R_n and a string resistance of 20,000 ohms, there should be with a nerve of 85,000 ohms resistance and a string of 12,000 ohms, a 40-fold amplification. In practice it was found that a maximal stimulus sent the shadow of the string off the film in every observation with the electron tube. The lack of constancy in the condition of the nerve made impossible any accurate quantitative comparisons in the case of submaximal stimuli, but as nearly as such comparison could be made it indicated about 45-fold amplification.

Records made in this experiment with and without the electron tube are shown in figure 23. In the first pair, (submaximal responses

produced by make shocks) the stimuli were of the same strength. In the second pair, since the maximal stimuli with the electron tube caused the excursion to exceed the width of the film, the response to a slightly submaximal break shock with the tube is compared with a maximal response with the unaided galvanometer. The speed of film is the same in all, but since the tuning fork causes interference with the electron tube arrangement, time is only shown on the records taken without it. It is noteworthy that the duration of the electrical disturbance in the nerve is much greater than one would suppose from the records made with the unaided galvanometer, amounting to as

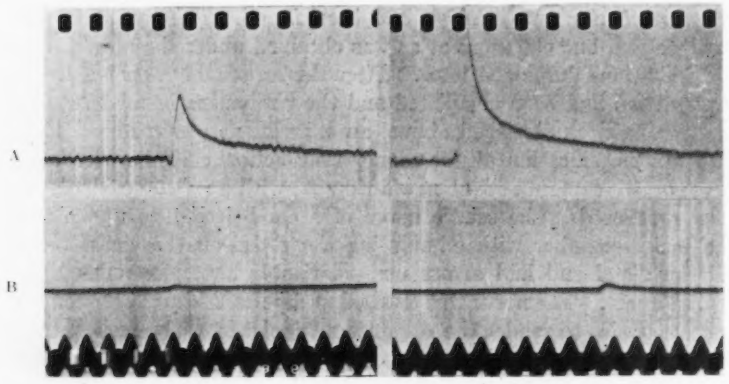


Fig. 23. Action currents in frog sciatic nerve (see text). String *H*. Tension, 1 cm. = 4.16×10^{-7} amp. A, electron tube arrangement; B, standard arrangement. Speed of film the same in all.

A1:—I_A, 1.10; E, 300; E_b, 189; R, 60,000; E_c, − 1.5. Stimulus submaximal make shock.

A2:—I_A, 1.10; E, 300; E_b, 207; R, 50,000; E_c − 1.5. Stimulus, break shock, 8.0 Z units (Martin scale).

B1, Stimulus, make shock of same strength as A1. B2, Break shock, 34.5 Z units.

much as a tenth of a second. The observations were made at ordinary room temperature, i.e., about 20°C. In this experiment and in another under similar conditions, every action current recorded, whether produced by a weak or strong stimulus, showed this surprisingly long duration. These experiments were performed in December and January with one of the original D-tubes mounted rigidly in the

open air. Three experiments performed on frog nerves in late February and early March failed to show any such prolonged duration (see fig. 26 A). The experimental conditions were identical except that the new D-tube already mentioned, having slightly different characteristics, had been substituted for that used in the earlier experiments (this being broken), and the tube was now mounted in the soundproof box. Tests with an artificial source of current fail to reveal anything in the new tube or its mounting which could account for this great change in the duration of the recorded action current. We are therefore led to the view that the difference in duration is physiological, possibly due to some seasonal change in the condition of the nerve.

It should also be noted in figure 23 that the direct action of the induction shock ("escape of current") appears in each record, preceding the action current (8, pp. 186–198). This was the case in all experiments with frog nerves even when the stimuli were subminimal. From its absence in the case of a nerve of low resistance (see fig. 26 B) we may infer that the prominence of this effect depends on a high resistance in the grid circuit.

In figure 24 are shown several electromyograms with and without the electron tube, some made with string *B*, a string of small resistance and large inertia, and some with string *H* having large resistance and small inertia. In the later of the two experiments when the high resistance string was used, care was taken to see that the contractions in each corresponding pair of observations were of the same strength, as indicated by the readings on the dynamometer which the subject gripped.

EXTRANEOUS OSCILLATIONS

In figures 23 and 24 it will be seen that those records which were made with the electron tube and string *H* manifest fine oscillations at a frequency of about 305 a second. These are superimposed upon the coarser oscillations due to physiological activity. Similar oscillations were found in some of the earlier experiments with string *G* (also one of small inertia) as well as with string *H*, when used with the electron tube and an artificial source of current, but they were largest in the experiments with the frog's nerve. They were absent in all experiments made with the heavy string, *B*. On the other hand it is evident that they are not mere mechanical vibrations of the lighter strings, for they are only evident when the galvanometer is used in connection with the electron tube.

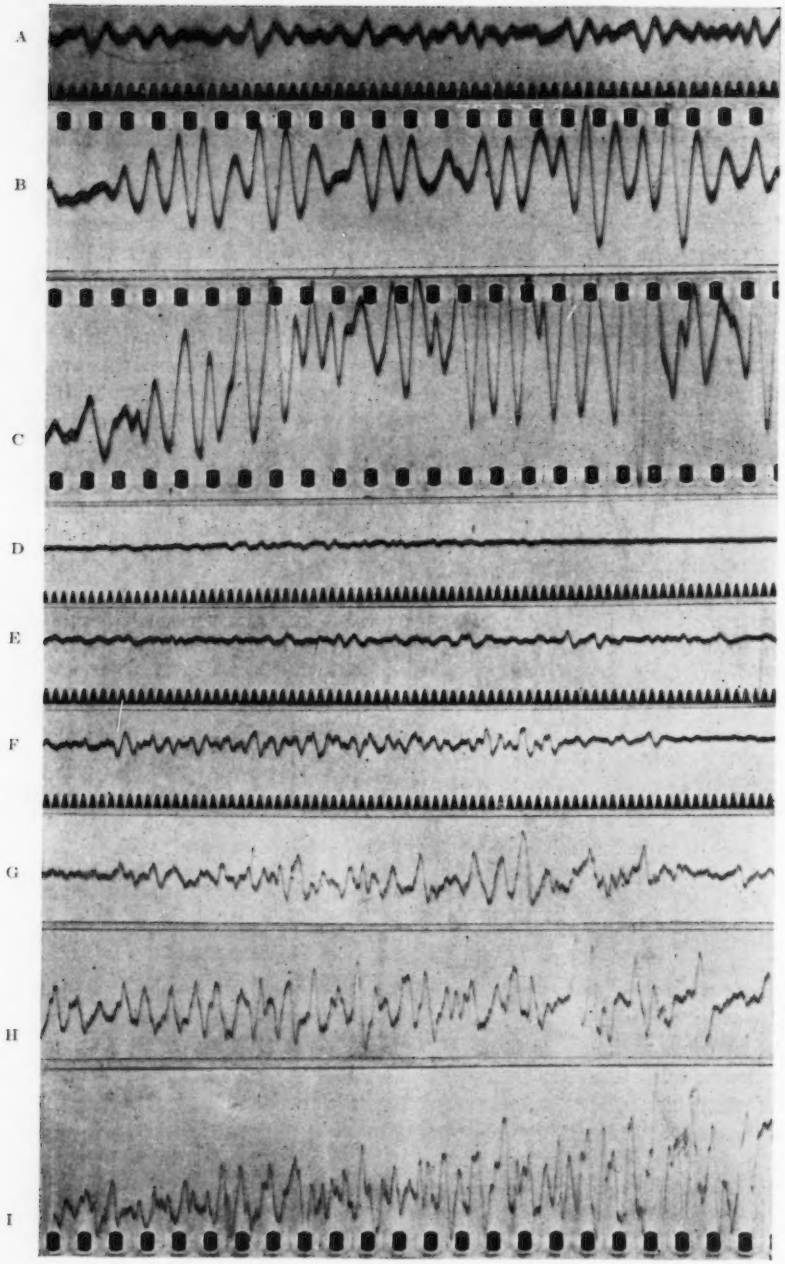


FIG. 24

A careful search was made for the cause of this disturbance. Since the electron tube arrangement had proved so sensitive to induction from the 110-volt motor used to drive the film in the older camera we were led to suspect that induction from other motors in the building might be the cause. Records were taken with all other motors in the building running and with all shut down, and the oscillations persisted under both conditions. The 12-volt motor which moved the film in the new camera was suspected. Records were therefore taken in which this motor was shut off while the clutch was in and the shutter open, so that the film continued to move as the motor slowed down. Still the oscillations persisted. The only significant fact noted in these experiments was that the oscillations were larger when a large resistance was included in the grid circuit, as was of course the case when the action current of the frog's nerve was being recorded. Finally, it was suggested to us by Dr. H. B. Williams that the cause of these oscillations might be modifications of the electron stream due to vibrations of one or more of the elements in the tube; filament, grid and plate. Such vibration is apt to result in these tubes from mechanical jarring or sound waves. This point was tested by tapping the tube gently while the film was being exposed; also by the sound of the human voice singing on the pitch indicated by the oscillations in the record; that is, about 305 a second. For comparison the sound of the voice singing on a pitch of 260 vibrations a second was tried. The effects of these three procedures are shown in figure 25. Evidently the tube has just such a natural period of vibrations and is extremely sensitive to mechanical jarring. It is also rather highly resonant to sound waves of that frequency.

In one experiment these vibrations were reduced to insignificant dimensions by greatly increasing the tension of the string and applying Einthoven's method of damping with a condenser across the string terminals (6). This remedy would answer fairly well for certain purposes, but it involves decreasing the excursion resulting from a given disturbance. Also it fails to give complete abolition of the oscillations.

Fig. 24. Electromyograms of human forearm flexors in voluntary contraction, subject squeezing dynamometer. A, B and C, string *B;* tension, 1 cm. = 5.25×10^{-7} amp. All others string *H;* tension, 1 cm. = 4.16×10^{-7} amp. A, strong contraction (strength not recorded), standard arrangement. B and C electron tube; I_A, 1.25; E, 238; R, 90,000; E_c, − 3.0. B, weak contraction (10 kilos); C, strong contraction (28 kilos). D, E and F standard. G, H and I, electron tube:—I_A, 1.10; E, 300; E_b, 207; R, 50,000; E_c, − 1.5. D and G, dynamometer reading, 6 kilos; E, 10 kilos; H, 9.5 kilos; F and I, 20 kilos.

On the advice of Dr. H. B. Williams, a sound-proof box was constructed to contain the electron tube, and was suspended by the method of Julius (7). The box was made large enough to contain the tube with an air space of an inch or two between it and the lining. It was made of $\frac{7}{8}$ inch wood, lined with $\frac{1}{8}$ inch of lead and an inch of acoustic felt inside of that, except on the bottom where the socket was secured directly to the lead lining. Holes were drilled in the sides of the box to admit leads of flexible stranded bell-wire, which were soldered to the terminals of the tube socket. Outside of the box these leads were soldered to insulated and lead-sheathed wires by which the tube

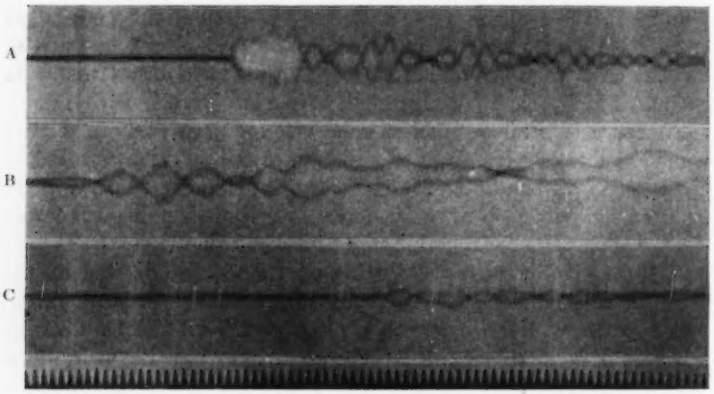


Fig. 25. Oscillations due to vibration of electron tube elements. String *H*. Tension 1 cm. = 4.16×10^{-7} amp. Condenser method. I_A, 1.10; E, 300; R, 50,000; E_c, -1.5. A shows effect of gently tapping tube. B, human voice singing on pitch of 305 a second, about a foot from tube. C, singing 260 vibrations a second, about the same intensity as in B. Time indicated as usual below.

was connected with the rest of the system, still in accordance with the wiring plan shown in figure 17, except that the milliammeter was eliminated as being unnecessary in the permanent installation. The lead lining of the box and the lead sheaths of the wires were grounded. The box was suspended by means of four steel springs such as are used for screen doors, attached to screw-eyes in the sides of the box at approximately the level of the center of gravity. The springs were hung from a bench secured solidly to the outside wall of the building, just under the galvanometer.

When the tube had been mounted in this box thus suspended, the same procedures that had caused the large oscillations shown in figure 25 were repeated. Loud sound waves from the human voice at a frequency of about 300 a second failed to produce any perceptible oscillations in the light string, *H*, which had vibrated so vigorously before. Screening from sound was evidently complete. The top of the bench directly over the suspension of the box was rapped vigorously, and this produced small vibrations whose amplitude was only a small fraction of that of the vibrations produced previously by rapping much more gently the table on which the tube had originally been mounted. When neither of these procedures was tried, the string appeared wholly free from the characteristic oscillations, whereas these had previously persisted, probably as a result of building vibrations, throughout the greatest quiet obtainable. The new mode of protecting against vibrations was thus found to be quite satisfactory.

More experiments were then carried out with both frog and cat nerves. In the case of frog nerves, having in circuit resistances of over 100,000 ohms, oscillations were still found, but they were much smaller than before the tube was protected, and they were irregular, having frequencies between 190 and 300 per second, with finer oscillations of about 600 per second superimposed. In the case of the sciatic nerve of a cat, the resistance of the part in circuit being about 10,000 ohms, the oscillations are almost if not wholly eliminated.

Figure 26 shows maximal monophasic action currents in both frog and cat nerves recorded with the electron tube in the sound-proof box. In each case there is shown for comparison the response of the same nerve to the same stimulus recorded with the unaided galvanometer. Calibration curves show the excursion of the string in each case with the same tension and with the same resistance in series when a constant voltage is applied. The irregular oscillations in the experiment with the frog's nerve may be seen in the figure.

Apparently the remaining oscillations are dependent on a large resistance in the grid (input) circuit. In seeking further light on their nature it was found that fairly regular oscillations of about 220 per second appeared whenever a large resistance, R_n, (e.g., 40,000 ohms) was introduced into the grid circuit as in figure 11. This is the same frequency with which the brushes shift contact on the commutator segments in the power house generator which supplies 110-volt direct current to the power mains throughout the building. Induction from this source is the probable cause of these oscillations. They are much

smaller than those which were traced to vibration in the tube. If the same resistance was introduced in the filament side of the input circuit, and if the greater part of R_1 lay on the opposite side of the key from the point at which the input circuit is led off, there was a brief excursion of the string in the opposite direction from that usually seen, following the closure of the key and preceding the usual excursion such as is shown in figure 14. This initial excursion is due to a capacity effect which need not be analyzed here. It has no bearing on the recording of action currents. But it is mentioned because of the confusion which may result if the resistance used to determine whether the negative grid potential is sufficient to prevent the absorp-

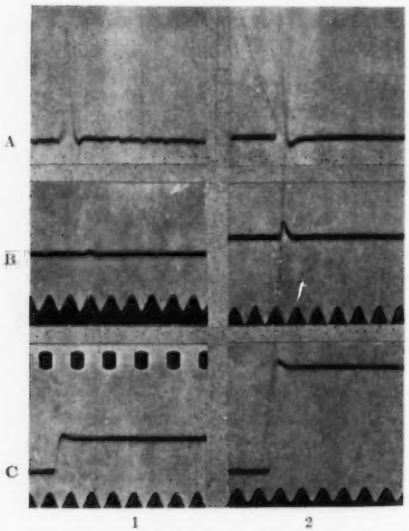


Fig. 26. Action currents recorded with tube in sound-proof box. String *H*. A, electron tube; B, standard; C, calibration curves.

1, frog sciatic nerve, resistance 115,000 ohms. Tension, 1 cm. = 8.3×10^{-7} amp. Stimulus in each case, 14.6 Z units.

2, cat sciatic nerve, resistance about 10,000 ohms. Tension, 1 cm. = 8.3×10^{-7} amp. Stimulus in each case, 46.2 Z units.

A1, I_A, 1.17; E, 300; R, 70,000; E_c, − 3.0.

A2, I_A, 1.06; E, 300; R, 70,000; E_c, − 3.0.

C1, $\frac{60}{S + 100{,}000}$. C2, $\frac{30}{S + 10{,}000}$.

Amplification in 2 is somewhat reduced by low value of I_A.

tion of current, is introduced in this side of the grid circuit, and if the primary resistance, R_1, is arranged in two parts with the key between them as was the case in most of our experiments.

The oscillations persisting in the records of frog nerves are probably in part due to the induction from the generator just mentioned. Their irregularity may indicate some further disturbance of physiological origin, or may be due to some extraneous cause not yet identified. At all events, the oscillations persisting in spite of the sound-proof box are insignificant as compared with a maximal action current in a nerve trunk; and with a resistance as low as is common in mammalian experiments they are imperceptible.

PRECAUTIONS

Several precautions in the use of the electron tube should be enumerated and emphasized. The most important of these concerns the avoidance of charging or discharging the condenser through the string. The rule is not to open or close any switch in the filament, grid or plate circuit, in short, anywhere in the electron-tube system, nor to make any adjustment in any part of the system while it is connected with the string galvanometer. All adjustments of current and voltage connected with the tube, and of by-pass resistance, should be made while the switch leading to the string is open and while the protective shunt (or equivalent short-circuit) is closed to enable the condenser to be charged from the B-battery. After all adjustments are made the switch leading to the string may be closed, and finally the protective shunt opened; but it should not be opened until at least 5 seconds after the closure of the switch which permits the charging of the condenser, for on account of the large resistance through which the charging current must pass, this time is required for the current to subside to a value which is safe to pass through the string. The string should be disconnected again before any switches in the rest of the system are opened. The least neglect of this rule will probably result in the loss of the string. There is one exception; the mere closing of the protective shunt, without disconnecting the string, is sufficient protection during minor adjustments of the filament current if made with sufficient caution.

If there is any possibility that one of the electrodes may be dislodged from its contact with the tissue under observation during an experiment, a shunt should be provided to prevent the grid circuit from

being broken. If a grid "bias" is used, the change in tube resistance which would result from breaking the grid circuit, would cause a current in the string so great as probably to destroy it. As a safeguard a graphite pencil line of about a megohm resistance, drawn on paper, has been connected across the leads to the non-polarizable electrodes, and has been found by experiment not to reduce measurably the excursion resulting from the action current of a nerve.

The filament current should be shut off when the tube is not in use. With the tube out of sight in a sound-proof box, one is apt to overlook this at the close of an experiment. The life of a tube is usually long, but not unlimited. In one case a tube was permanently impaired by accidently leaving this current on until the battery was discharged.

Other precautions have been touched on in connection with the electrically induced oscillations caused by the Sandstrom kymograph motor and by the electrically driven tuning fork, and in connection with the oscillations traceable to vibration of the tube. Two distinct classes of disturbance are met with, those electrically induced and those of a mechanical origin. Two ways of dealing with the electrical disturbances are open,—prevention of the cause, and intervention between the cause and effect by shielding. It is desirable to avoid the use of electric motors in the vicinity of the system, and to minimize sparking from any source. To drive the film a low voltage motor completely encased in grounded metal is satisfactory. To record time a device which is free from the sparking of an electromagnetic tuning fork, should be used.

It has been found that rapid motion of the body close to the grid circuit, or shuffling of the feet produces marked excursions of the string. These are to be explained by the electrostatic induction in the grid circuit by the more or less charged body. On this account the experimenter should stand as still as possible during an observation. This disturbance is somewhat reduced by grounding the body of the experimenter. This has been effected by keeping the hand on the clutch which sets the film in motion, and which is grounded.

If, when a stimulating inductorium is set up near the electron tube system, the primary circuit of the inductorium is touched with the hand, an excursion of the string will result when the circuit is made or broken, even if there is no connection between the inductorium and the recording system (see p. 428). It is essential, therefore that if the stimulating key be operated by hand it should be done through an insulated handle.

It is important to see that all connections are good; they should be soldered as far as possible. A loose connection will introduce serious disturbance and may result in the loss of a string.

It is also very important that all batteries, especially the B-battery, should be in good condition. Dry cells which are nearly exhausted or storage cells in bad condition or bubbling as a result of too recent charging, may cause troublesome excursions of the string.

The danger of mistaking static effects or other extraneous disturbances for physiological effects is always present in the study of action currents with the string galvanometer. Amplification with the electron tube enormously increases this danger; one cannot be too careful to guard against it both by eliminating all possible sources of disturbance and by scrutinizing all results obtained with reference to latent period, dependence on vitality in the tissue studied, and any other possible criteria which may serve to verify the physiological origin of the current observed.

SUMMARY

1. The electron tube is a device wherein a stream of electrons emitted from a hot cathode in a vacuum and drawn to an anode (plate) by a high voltage battery, is modified by small changes of potential applied to a third electrode known as the grid, placed in the intervening space. In this way it serves as an amplifying relay and is commonly used as such in radio telegraphy.

2. This device offers great possibilities of amplifying such action currents in the nervous system as are too small to be recorded satisfactorily with the string galvanometer alone. When the string galvanometer is used to record the current amplified by this device it cannot be placed directly in the path of the high voltage current, as is done with the telephone, without destruction of the string. This must be protected from the direct current, and yet so connected with the system as to record changes in the electron stream.

3. Three methods of connecting the string with the tube in such a way as to afford the needed protection, were tried. These are designated the transformer method, the bridge method and the condenser method. They are illustrated diagrammatically in figures 3, 4 and 5. The condenser method was the only one which proved wholly satisfactory. It consists in placing a very large condenser in series with the string, and shunting the plate current by both through a resistance

of the same order of magnitude as the plate-to-filament resistance of the tube. By using a condenser with a capacity of as much as 15 microfarads the distortion due to gradual discharge of the condenser when the system is unbalanced becomes negligibly small in the case of action currents of brief duration. A permanent wiring arrangement was installed by means of which the electron tube system can be made available for the amplification of action currents in a tissue under observation, by merely throwing a few switches and making two or three adjustments, a process requiring less than two minutes. "D-tubes" loaned by the Western Electric Company, have been used in these experiments.

4. An effort was made to determine those values of filament-heating current, plate-battery voltage, grid potential, and by-pass resistance which would afford the greatest possible amplification. In general, amplification depends on a large proportional change in tube resistance for a given change in grid potential, together with other factors whose interaction is rather complex.

5. Complete mathematical analysis of the problem, using the empirically established formulae for tube characteristics, leads to insoluble expressions. Dr. E. L. Chaffee devised a method of obtaining an approximate formula for expressing in terms of two easily measurable quantities in the tube characteristics and of the resistance of the string and the by-pass resistance, amplification in any given case. The formula is $\frac{di_s}{dE_c} = \frac{\mu R}{(R+S)r + RS}$. In this i_s is the current in the string; E_c is grid potential; R is the by-pass resistance; S is the resistance of the string; r is the virtual resistance of the tube as determined by the reciprocal of the slope of the curve correlating plate current with plate potential; μ expresses the sensitiveness of plate current to grid potential as compared with its sensitiveness to plate potential, in other words, the ratio between the slopes of the curves correlating plate current with grid potential and with plate potential respectively.

6. A large number of experiments in which the amplification was measured with a large variety of combinations of the quantities above enumerated, verified Doctor Chaffee's formula. Unless the grid is given an initial negative potential the grid circuit will absorb current and thereby detract considerably from the amplification. It was found that best results are obtained with a filament current of 1.1 to 1.2 amperes, with a negative grid potential of 1.5 to 3.0 volts (the larger value

being necessary with some tubes), with a plate voltage of approximately 200, and with a plate battery voltage as large as is convenient to install in the laboratory, the difference between plate and plate battery voltage being consumed in the by-pass resistance. This resistance should be at least half as large as the resistance of the tube, and preferably more than that. In practice the combination should depend on preliminary tests of the individual tube. Convenient and efficient arrangements with the D-tubes used have been within the following limits—filament current 1.1 to 1.2; grid potential −1.5 or −3.0 (one or two dry cells); plate battery voltage 300; by-pass resistance 50,000 to 80,000 ohms.

7. The use of paper condensers, which are most convenient when a very large capacity is desired, introduces sufficient leakage of current to cause a considerable displacement of the string. With the Cambridge string galvanometer the string may be simply brought back into the field by lateral adjustment of the fiber case. A somewhat better method is to tap off from a dry cell through a high resistance an equal and opposite difference of potential.

8. As nearly as we could measure it, the amplified current in the string galvanometer was strictly proportional to the e. m. f. applied to the grid circuit.

9. If the grid potential is kept negative with respect to the filament by a sufficient amount to prevent the flow of current in the grid circuit, the excursions of the string depend wholly on the e. m. f. impressed on the grid, and are independent of the resistance of the input circuit, i.e., with values of resistance at least up to 150,000 ohms. In this way the system operates as an electrometer rather than a galvanometer. The amplification, or ratio between excursions of the string with and without the tube depends both on the resistance of the input or tissue circuit and on the resistance of the string. The electron tube necessarily introduces high resistance into this system; therefore if the current to be recorded arises in a tissue of small resistance, and is recorded with a string of small resistance, the use of the electron tube will afford no amplification. But if either string or tissue has a large resistance there will be amplification. Given the resistance of the string and the amplification obtained with any given tissue resistance, the amplification obtainable with any other tissue resistance may be simply calculated. In the case of a frog's sciatic nerve having a resistance of 100,000 ohms between the leading-off electrodes, the amplification may be more than 50-fold.

10. Records have been made with the electron tube of the action currents in frog's nerve, and in human muscles during voluntary contraction, both with a high resistance string and with a low resistance string. These have been compared with action currents recorded directly with the unaided galvanometer under otherwise similar conditions. In the case of the frog sciatic nerve an amplification of more than 40-fold has been found.

11. When a motor operated on the 110-volt power mains was used to drive the film for photographic recording, oscillations were induced in the galvanometer through the electron tube system, which we were unable to prevent by screening. It proved necessary to record with a camera designed to work with a 12-volt motor completely encased in metal and run from a storage battery within the room. This camera has certain advantages over those previously used, and a brief description is included. Oscillations were also induced by an electrically driven tuning fork. These oscillations were probably traceable to high frequency currents arising in the electric spark and rectified by the tube. It was necessary to dispense with this method of recording time.

12. Other oscillations occurred in the galvanometer when connected with the electron tube system, which were traceable to vibrations in the elements in the tube, causing fluctuations in the electron stream. This difficulty was eliminated by installing the electron tube in a sound-proof box, suspended by the method of Julius. Smaller oscillations persisted in experiments with nerves of very high resistance, traceable probably to induction from the power mains. They are so small as to be insignificant, and were absent in experiments with tissues of medium resistance.

13. In addition to the precautions indicated in the last two sections, it is most important that the string be protected from the high voltages used in this system by careful avoidance of adjustments in any part of the system while the string is connected with it, and by allowing the condenser to become charged through a protective shunt or short-circuit before any connection with the string is made. Other confusing effects due to static electricity or bad connections or batteries in poor condition, must be carefully guarded against, if satisfactory results are to be obtained.

We wish to express our thanks to Dr. H. B. Williams, Dr. H. B. Arnold, Mr. E. H. Colpitts, Mr. S. W. Dean, Mr. Sewall Cabot and

Prof. G. W. Pierce for suggestions and advice, and especially to Dr. E. L. Chaffee for the mathematical analysis, and to Miss M. D. Ring for help in preparing the work for publication.

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VARIATIONS IN THE RESPIRATORY DEAD AIR SPACE DUE TO CHANGES IN THE DEPTH OF BREATHING

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The variation which occurs in the capacity of the dead air space of the lungs because of changes in the tone of the bronchial muscles or in the amount of air inspired at each breath, is unsettled. In a previous paper of this series (1) it was shown that the dead air space has a fairly constant volume at ordinary depths of inspiration, and the great variation in the capacity of the dead air space found by Haldane (2) and by Henderson (3) and co-workers was not sustained, while the findings of Krogh and Lindhard (4) were in the main substantiated. These latter observers found evidence of a small increase in the capacity of the dead air space when maximal breaths were taken. While we were unable to obtain absolute evidence of even this small increase, we believe that our early results suggested such a possibility. Our experiments were free from many errors present in earlier work, but owing to the manner of combining our figures we obtained averages of the dead air space for all depths of inspiration. Later work by us indicated that, at moderate volumes of inspiration, the dead air space was a relatively fixed volume. Unfortunately, in the method employed we did not and could not use extreme volumes of inspiration. Moreover the spirometers we used had a dead air space of their own which, although we tried to eliminate it by filling with expired air before the observation, must have contributed toward making our results too high. In order to put this subject to further test, we have devised a method which we believe will, if properly carried out, give a fairly accurate measure of the capacity of the air passages at all depths of inspiration.

In this method, as in the former method, we used the mathematical formula given in our first paper (5). By it the carbon dioxide contents of a large and a small expiration following a measured inspiration are compared with the volume of air in a large and a small expiration in

a manner so that the air from the dead space may be determined. In the application of this formula to estimating the capacity of the dead air space at different lung volumes, we assume that the amount of carbon dioxide which is picked up by the definite volume of air inspired and expired quickly, following a forced expiration to residual air, is fairly constant in amount for a given state of bodily activity. That this is the case we have satisfied ourselves. We also assume that the carbon dioxide in the alveolar air collected for analysis has a uniform concentration. We admit that such is not the case actually, but for practical purposes of the experiment, providing the expirations compared do not differ by more than 400 cc., the assumption may be allowed.

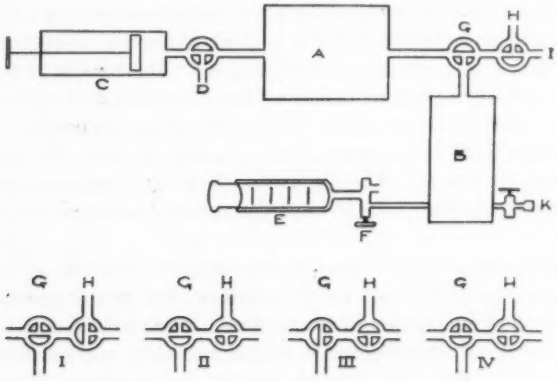

FIG. 1

It is also assumed that, given the same volume of inspiration, the capacity of the air passages will be the same in the different observations necessary to collect the required data. By progressively increasing the amount of air inspired, any variation of the dead air space due to stretching of the bronchial tree can be detected if such variations be greater than the experimental error of the method. The essential feature of this method is that a sharp, quick expiration to residual air be followed by a quick inspiration of a measured volume of air, and this followed by a quick expiration, the first portion of which is collected for measurement and analysis. The amount of air collected must be varied between 400 and 800 to 1000 cc. for best results.

Briefly the experimental procedure is as follows. Reference is to figure 1.

Bag *A* is a flat rubber bag capable of holding 5 liters of air. It connects with a three-way stopcock, *G*, which in turn connects with stopcock *H*, which has a mouthpiece, *I*. Bag *B* is a flat rubber bag of 1200 cc. capacity attached to cock *G*. *C* is an automobile grease gun, delivering 340 cc. of air at a stroke, and *D* a three-way cock for taking air into the gun and delivering it into bag *A*. *E* is a 100 cc. Luer syringe, and *F* a three-way cock attached to bag *B* for measuring the volume of air in bag *B*. *K* is a tube with cock for obtaining samples for analysis. The bags are flat and do not hold an appreciable amount of air when evacuated.

Bag *A* is filled with a measured amount of air by *C*. The cocks are then as shown in position 1. The subject is seated, and after five minutes of rest makes a quick forced maximal expiration. This must have followed a normal inspiration. Cock *H* is then turned to position 2, and a quick inspiration empties bag *A*. The breath is held while cock *G* is turned to position 3 and a forced expiration is made into bag *B*. In this case, however, cock *G* is quickly turned to position 4 during the expiration. By this means it is possible to limit the amount of air collected in bag *B*. The air in bag *B* is then analyzed accurately for its carbon dioxide content, and its volume measured by means of syringe *E*.

For each inspiratory volume it is necessary to make at least four observations, in which two small volumes and two somewhat larger volumes of the first portion of the expired air are collected in bag *B*.

The amounts of air collected in bag *B* and their carbon dioxide content are combined in the formula for the estimation of the dead air space as given in our previous papers. The formula reads: Volume of large multiplied by the volume of the small portions of expired air, multiplied by the difference in the percentage of carbon dioxide in the large and small portions of expired air, divided by the difference in the total volume of carbon dioxide found in the large and small portions of expired air, equals the volume of the dead air space.

The validity of using this method for obtaining the volume of the dead air space depends on certain factors: *a*, For each individual series of observations in which a given volume of air is inspired, the amount of carbon dioxide produced per unit of time must be constant during the experiment. For this reason it is absolutely necessary that the subject rest 3 to 5 minutes before each observation. *b*, The inspiration of the measured amount of air in the large bag must follow the deepest possible expiration, which in turn must have followed a normal inspira-

tion. This must obtain, since otherwise the amount of carbon dioxide in the lung air and the amount which is delivered by the blood to the lung air during the moment of observation would vary. *c*, The small bag, which collects the first part of the inspiration, must not contain any air except that which has been received from the first part of the forced expiration. *d*, The analysis of the carbon dioxide content of the air in the small bag must be very accurate, and the volume of the air collected in the small bag must be measured very accurately. *e*, The periods of time taken for the observations must be about equal, in order that the carbon dioxide eliminated by the blood be the same for each observation.

The results obtained on the subjects are given in tables 1, 2, 3 and 4, and are plotted as curves on cross-section paper, figure 2, the abscissae of which represent the volumes of air inspired and the ordinates the volumes of dead air space found with the respective inspiratory volume. It will be noted that when ordinary volumes of inspiration—1500 to 2500 cc. of air—are taken after a forced expiration (which amount is practically that which is ordinarily present in the lungs at the end of the normal inspiration), the variations of dead air space are very little but progressively increase; while with larger volumes of inspiration the dead air space increases from 100 to 115 per cent maximum. In other words, the increase in dead air space with the increasing volumes of inspiration is not a linear function to the depth of inspiration but a powered function. The explanation of this phenomenon no doubt rests in the fact that the small bronchioles contribute much to the volume of dead air space and are expanded somewhat during the inspiratory effort. The greater the inspiratory effort, the greater must be the negative pressure of the intra-thoracic pressure. Since the bronchi and bronchioles are made up of more or less elastic tissue, it is reasonable to suppose that their walls are dilated more or less proportionately to the degree of intra-thoracic pressure. Since the area of a circle varies as the square of its radius, it is apparent that a slight progressive increase in the diameter of the bronchioles will cause a relatively disproportionate and larger increase in the area of the cross section of the bronchioles.

The curves obtained very strongly suggest that this condition obtains to a greater or less degree during deep breathing. It is interesting to note that the maximum variation of dead air space, obtained when the deepest possible inspiration was made, is about 115 cc., corresponding closely to the figures obtained by Krogh and Lindhart (4). It

TABLE 1

Subject R. G. Pearce, height 5 feet 9 inches, weight 176 pounds. Vital capacity about 4000 cc.

EXPERIMENT NUMBER	AIR INSPIRED FROM BAG *A*	AIR EXPIRED INTO BAG *B*	CO_2 IN BAG *B*	VOLUME OF CO_2 IN BAG *B*
	cc.	*cc.*	*per cent*	
1	1700	660	3.50	23.00
2	1700	690	3.55	24.50
3	1700	390	3.10	12.10
4	1700	350	3.00	10.50
1	2380	685	2.90	19.80
2	2380	590	2.80	16.50
3	2380	303	2.25	6.80
1	3060	585	2.60	15.25
2	3060	383	2.15	8.20
1	3740	720	2.40	21.70
2	3740	655	2.85	18.60
3	3740	665	2.80	18.50
4	3740	395	2.10	7.80
5	3740	365	2.10	7.30

When 1700 cc. of air are inspired:

By combining 1 and 3 we have 94.5 cc. dead air space
By combining 1 and 4 we have 92.0 cc. dead air space
By combining 2 and 3 we have 97.5 cc. dead air space
By combining 2 and 4 we have 95.0 cc. dead air space
Average dead air space when 1700 cc. are inspired is 94.8 cc.

When 2380 cc. of air are inspired:

By combining 1 and 3 we find 104 cc. of dead air space
By combining 1 and 4 we find 101 cc. of dead air space
Average dead air space when 2380 cc. are inspired is 102.5 cc.

When 3060 cc. of air are inspired:

By combining 1 and 2 we have 143 cc. of dead air space

When 3740 cc. of air are inspired:

By combining 2 and 3 we find 201 cc. dead air space
By combining 3 and 5 we find 182 cc. dead air space
By combining 1 and 4 we find 184 cc. dead air space
By combining 1 and 5 we find 165 cc. dead air space
By combining 3 and 4 we find 196 cc. dead air space
By combining 3 and 5 we find 172 cc. dead air space
Average dead air space when 3740 cc. are inspired is 183 cc.

Summary

1700 cc. inspired gives dead space of 94.5 cc.
2380 cc. inspired gives dead space of 102.5 cc.
3060 cc. inspired gives dead space of 143.0 cc.
3740 cc. inspired gives dead space of 183.0 cc.

TABLE 2

The following results were obtained on D. H. H. between 4:00 and 6:00 p.m., June 3, 1918, the subject being seated in a chair at rest. The experiment was the same as above, save that the expired air was collected in a small spirometer. The dead space of the apparatus when these data were collected was probably 20 cc. greater than with the improved method using the rubber bag B. His vital capacity was about 4000 cc.

EXPERIMENT NUMBER	INSPIRED	EXPIRED SPIROMETER *B*	CO_2 SPIROMETER *B*	CO_2 SPIROMETER *B*
	cc.	*cc.*	*per cent*	*cc.*
1	1500	560	2.75	15.40
2	1500	525	2.70	14.20
3	1500	930	3.05	28.40
4	1500	1035	3.10	32.10
1	2100	735	2.70	19.80
2	2100	670	2.60	17.40
3	2100	1070	3.05	32.60
4	2100	1085	3.10	33.60
1	2900	1130	2.50	28.20
2	2900	1060	2.47	26.20
3	2900	500	1.80	9.00
4	2900	525	2.00	10.50
5	2900	525	1.85	9.70
1	3600	1035	2.20	23.20
2	3600	1125	2.32	26.10
3	3600	1105	2.33	26.80
4	3600	620	1.75	10.85
5	3600	630	1.75	11.00
1	4300	680	1.85	12.60
2	4300	675	1.75	11.80
3	4300	1125	2.25	25.30
4	4300	1085	2.15	23.30

When 1500 cc. of air are inspired:

By combining 1 and 4 we find 120 cc. dead air space
By combining 2 and 4 we find 121 cc. dead air space
By combining 1 and 5 we find 130 cc. dead air space
By combining 2 and 4 we find 121 cc. dead air space
Average dead air space when 1500 cc. are inspired is 123 cc.

When 2100 cc. of air are inspired:

By combining 1 and 3 we find 120 cc. dead air space
By combining 2 and 3 we find 129 cc. dead air space
By combining 1 and 4 we find 140 cc. dead air space
By combining 2 and 4 we find 139 cc. dead air space
Average dead air space when 2100 cc. are inspired is 132 cc.

When 2900 cc. of air are inspired:

By combining 1 and 3 we find 208 cc. dead air space
By combining 2 and 3 we find 206 cc. dead air space
By combining 1 and 4 we find 156 cc. dead air space
By combining 1 and 5 we find 208 cc. dead air space
By combining 2 and 5 we find 209 cc. dead air space
By combining 2 and 4 we find 166 cc. dead air space
Average dead air space when 2900 cc. are inspired is 192 cc.

When 3600 cc. of air are inspired:

By combining 1 and 4 we find 238 cc. dead air space
By combining 1 and 5 we find 245 cc. dead air space
By combining 2 and 4 we find 261 cc. dead air space
By combining 3 and 4 we find 245 cc. dead air space
Average dead air space when 3600 cc. of air are inspired is 247 cc.

When 4300 cc. of air are inspired:

By combining 1 and 3 we have 241 cc. dead air space
By combining 2 and 3 we have 280 cc. dead air space
By combining 1 and 4 we have 201 cc. dead air space
By combining 2 and 4 we have 253 cc. dead air space
Average dead air space when 4300 cc. of air are inspired is 243 cc. In this case 3800 cc. was about the vital capacity.

Summary

1500 cc. inspired gives dead space of 123 cc.
2100 cc. inspired gives dead space of 132 cc.
2900 cc. inspired gives dead space of 192 cc.
3600 cc. inspired gives dead space of 247 cc.
3800 cc. (maximum) gives dead space of 243 cc.

This is given in form of curve in which 20 cc. are deducted from each figure because of the dead space present in the spirometer. This figure is an approximate one only for the actual dead space of the Krogh spirometer is difficult to measure accurately.

TABLE 3

Subject R. G. Pearce

EXPERIMENT NUMBER	AIR INSPIRED FROM BAG *A*	AIR EXPIRED INTO BAG *B*	CO_2 IN BAG *B*	VOLUME OF CO_2 IN EXPIRED AIR
	cc.	*cc.*	*per cent*	
1	1700	830	3.05	25.40
2	1700	925	3.00	27.75
3	1700	560	2.80	15.70
4	1700	455	2.70	12.25
1	2680	720	2.30	16.55
2	2680	520	2.10	10.90
3	2680	470	2.00	9.40
4	2680	340	1.75	5.95
1	3740	750	2.30	17.40
2	3740	663	2.25	14.90
3	3740	430	1.75	7.55
4	3740	425	1.75	7.45

When 1700 cc. of air are inspired:

By combining 1 and 3 we find 120 cc. dead air space
By combining 1 and 4 we find 101 cc. dead air space
By combining 2 and 3 we find 85 cc. dead air space
By combining 2 and 4 we find 82 cc. dead air space
Average dead air space when 1700 cc. are inspired is 97 cc.

When 2680 cc. of air are inspired:

By combining 1 and 3 we find 142 cc. dead air space
By combining 1 and 4 we find 128 cc. dead air space
By combining 2 and 4 we find 125 cc. dead air space
Average dead air space when 2680 cc. of air are inspired is 132 cc.

When 3740 cc. of air are inspired:

By combining 1 and 3 we find 176 cc. of dead air space
By combining 2 and 3 we find 176 cc. of dead air space
By combining 1 and 4 we find 191 cc. of dead air space
By combining 2 and 4 we find 189 cc. of dead air space
Average dead air space when 3740 cc. of air are inspired is 183 cc.

Summary

1700 cc. inspired gives a dead air space of 97 cc.
2680 cc. inspired gives a dead air space of 132 cc.
3740 cc. inspired gives a dead air space of 183 cc.

TABLE 4

Table 4 gives the data collected in the case of Dr. M. D., who is 5 feet 9½ inches in height and weighs 130 pounds. His tidal air amounts to about 500 cc., and his complemental air to 1200 cc. When breathing in 2200 cc. following a forced expiration, his lungs would contain about 500 cc. more than at the end of a normal inspiration, or when breathing 3600 cc. as above, 1900 cc. more than is present at the end of a normal inspiration. His vital capacity was about 3600 cc.

EXPERIMENT NUMBER	AIR INSPIRED FROM BAG *A*	AIR EXPIRED INTO SPIROMETER *B*	CO_2 IN SPIROMETER *B*	AMOUNT CO_2 IN SPIROMETER *B*
	cc.	*cc.*	*per cent*	
1	2200	1000	3.55	35.5
2	2200	1000	3.50	35.0
3	2200	790	3.40	26.8
4	2200	700	3.30	23.1
5	2200	650	3.25	21.2
6	2200	400	2.75	11.0
1	3600	1500	3.00	40.5
2	3600	1050	2.90	30.4
3	3600	800	2.75	22.0
4	3600	575	2.40	13.8

When 2200 cc. of air are inspired:

By combining 1 and 6 we find 130 cc. dead air space
By combining 1 and 5 we find 132 cc. dead air space
By combining 1 and 4 we find 140 cc. dead air space
By combining 6 and 3 we find 121 cc. dead air space
By combining 6 and 4 we find 128 cc. dead air space
Average dead air space when 2200 cc. are inspired is 131 cc.

When 3600 cc. of air are inspired:

By combining 1 and 4 we find 185 cc. dead air space
By combining 1 and 3 we find 182 cc. dead air space
By combining 1 and 2 we find 195 cc. dead air space
By combining 2 and 4 we find 182 cc. dead air space
By combining 3 and 4 we find 196 cc. dead air space
Average dead air space when 3600 cc. are inspired is 184 cc.

Summary

2200 cc. inspired gives a dead air space of 131 cc.
3600 cc. inspired gives a dead air space of 184 cc.

is most important to note that variations in the dead air space in the ordinary volumes of respiration are slight, and this point makes it possible to determine the percentage composition of the alveolar air with reasonable accuracy by the examination of the gaseous content of the expired air, correction being made for the dead air space. It is also interesting to note that the volume of the respiratory dead air space thus determined is somewhat smaller than has hitherto been thought.

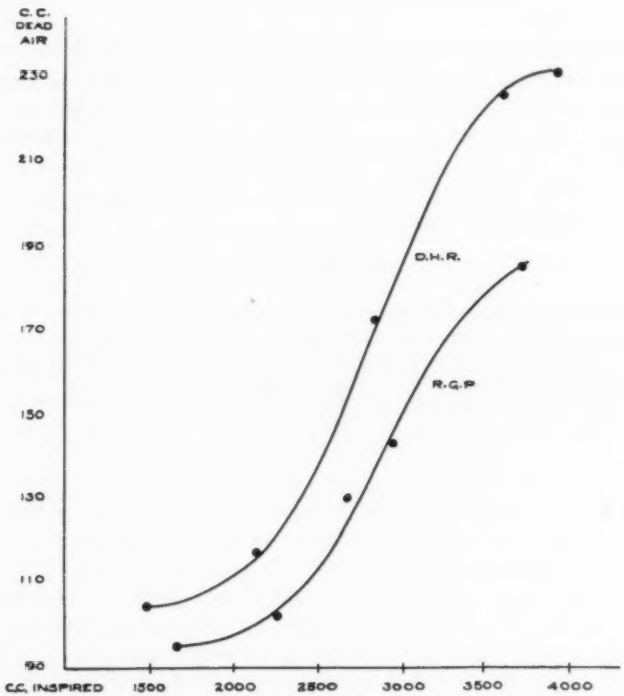

FIG. 2

It was hoped that evidence might be obtained of normal variations in the respiratory dead air space at different times of the day and under varying conditions. Such variation has been noted previously by us when the dead air space was determined by the method described in previous articles of this series. The experimental error of the method is probably too great to allow this to be done.

SUMMARY

1. A new method of determining the capacity of the dead air space is described.

2. The volume of the respiratory dead air space is relatively constant at all volumes of respiration.

3. The changes which occur in the volume of the dead air space when the variations in depth of breathing are such as ordinarily occur during moderate effort, are not of as great magnitude as when forced respiratory efforts are made.

4. The percentage composition of the alveolar air can be determined with approximate accuracy from analysis of the expired air and an assumed volume of the dead air space, if the depth of breathing is not excessive. This method is as reliable as the best direct methods now used for the determination of the percentage composition of alveolar air, providing the respiratory volume is large enough to completely wash out the dead air space.

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THE SUB-ARACHNOID AND INTRA-ARTERIAL ADMINISTRATION OF SODIUM BICARBONATE AND OTHER ELECTROLYTES

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INTRODUCTION

It has been shown that extensive voluntary hyperpnoea may produce mild tetany in the normal human subject (1). The causation of this effect was interpreted as a mild alkalosis due to the "washing out" of CO_2 from the blood at the lung surface. A certain amount of evidence in support of this view is given by the fact that on two occasions mild tetany was produced in dogs by large injections of sodium bicarbonate. It would therefore appear that such tetany is due either to a decrease in the C_H of the blood or else to a disturbance in the sodium, potassium, calcium equilibrium within the nervous tissue. In order to throw further light upon this subject a number of experiments were carried out on dogs, rabbits, frogs, and turtles, in which the effects of sub-arachnoid and intra-arterial injections of various electrolytes were studied. It has been shown by Weed and Wegeforth (2) that irrigation of the spinal and cerebral sub-arachnoid spaces with bicarbonate-free Ringer's solution can be carried out without serious effect, whereas irrigation with isotonic solutions of sodium chloride results in the manifestation of severe toxic effects. They found also, when the irrigation fluid was free from KCl that no noticeable toxic symptoms developed, while irrigation with an isotonic Ca-free solution resulted in the same toxic manifestations as when 0.7 per cent to 1 per cent NaCl in distilled water was used. The importance of the Ca ion in relation to physiological activity has frequently been emphasized (3). Locke (4) showed that the Ca ion is necessary for the transference of the excitory process from nerve to muscle and Overton (5) demonstrated that it is equally necessary for the transmission of the excitory

state through the synapse. It is very probable that a variety of effects which may be elicited in various ways, such as will be subsequently described, are in a large measure due to a disturbance in the Ca balance within the nerve cell.

METHODS

The effects of sub-arachnoid injections of various electrolytes were studied in both normal and anesthetised animals. Dogs were used for the most part but experiments were also carried out on rabbits, frogs, and turtles. The animal, in practically all experiments where graphic records were taken, was first placed under morphine-ether anesthesia, a cannula was then inserted in the left carotid artery and connected to the mercury manometer, tracheotomy was performed and anesthesia maintained by the ether bottle method described by Jackson (6). The respiratory movements were recorded by means of a Marey tambour which was connected by rubber tubing to a glass cannula which was inserted into the rubber tubing connecting the tracheal cannula with the valved top of the ether bottle. The valves in the ether bottle were so adjusted that uniform anesthesia was maintained, the tracing of the Marey tambour lever then being a relative index of the rate and depth of the respiratory movements. Two needles were, as a rule, placed in the spinal canal, one in the lumbar, the other in the subcerebellar region.

Injections into the sub-arachnoid space were made very slowly and cautiously and the volume of fluid injected at any one time was small as the records indicate. As a rule 2 or 3 cc. of spinal fluid (in large dogs) was drawn off before an injection was made or else the obturator was removed from the needle for two or three minutes prior to making the injection, and this latter was not replaced until a few minutes after the injection had been made. These precautions were taken in order to exclude the possibility of any effects elicited by spinal injection being due to increased pressure of the cerebro-spinal fluid.

Intra-cerebral injections were made through a needle placed in the third ventricle, a small opening having first been made in the parietal bone just lateral to the median plane.

Intra-venous and intra-arterial injections were also made. The external jugular vein was chosen for the former while the vertebral or carotid artery was selected for the latter.

Intra-spinal injections were made on rabbits after lumbar puncture had been performed under local anesthesia, while intra-spinal injections

were made in frogs and turtles by means of a small hypodermic needle which was placed through the occipito-atlantoid ligament. When dogs were not placed under morphine-ether anesthesia, a small amount of morphine was given by subcutaneous injection and then lumbar puncture was performed under local anesthesia.

RESULTS

Sub-arachnoid and intra-arterial injections of $NaHCO_3$. It was found that the injection of small amounts of sodium bicarbonate solution into the lumbar sub-arachnoid space resulted almost immediately in the manifestation of marked tetany of the musculature of practically the whole body, the muscles of the posterior half of the body being, however, more affected than those of the anterior region (protocols 1, 2 and 3). Experiments have been carried out on twenty dogs and six rabbits and in every instance where $NaHCO_3$ has been injected into the spinal canal in appreciable amounts, tetany has resulted. Tetanic convulsions were also elicited in frogs and turtles by intra-spinal injections of mere traces of $NaHCO_3$. The effect can be produced in both anesthetised and unanesthetised animals. The strength of the bicarbonate solutions used varied from 1 per cent to 10 per cent. Intra-spinal injections of $NaHCO_3$ in addition to producing tetany resulted in intensive and sometimes very much prolonged stimulation of the chief medullary centers which was manifested by a great increase in lung ventilation, rise in blood pressure and various degrees of cardiac vagus activity (fig. 1). If the $NaHCO_3$ were injected in sufficient amount to cause most intensive tetanic spasms, respirations were inhibited and artificial respiration had to be resorted to until the spasm had modulated to a sufficient degree to make spontaneous respiration possible. It was found that tetany could be antagonized to a considerable extent by the intra-spinal injection of $CaCl_2$ (fig. 1), while subcutaneous injection antagonized only to a slight degree. The medullary stimulation was, however, not counteracted to the same degree by the intra-spinal injection of $CaCl_2$. When injections of $NaHCO_3$ were made into the sub-cerebellar cistern after occipital-atlantoid puncture, the medullary stimulation was much more marked, while tetany was less readily obtained. If but a small quantity of $NaHCO_3$ was injected in this region there was no tetany but only violent hyperpnoea, increased blood pressure and evidence of cardiac inhibition (fig. 2). If the dose was slightly increased, in addition to the above symptoms mild

tetany of the muscles of the face, neck, shoulders, and fore limbs was produced, while a still larger injection caused the tetany to become general. Intra-cerebral injection of $NaHCO_3$ through a needle placed in the third ventricle produced somewhat similar effects as injection into the subcerebellar cistern (fig. 3, *e*). While intra-venous injections of comparatively large quantities of $NaHCO_3$ were without marked effect it could be shown that the medullary stimulation following the subarachnoid injection of $NaHCO_3$ was due in part at least to the direct

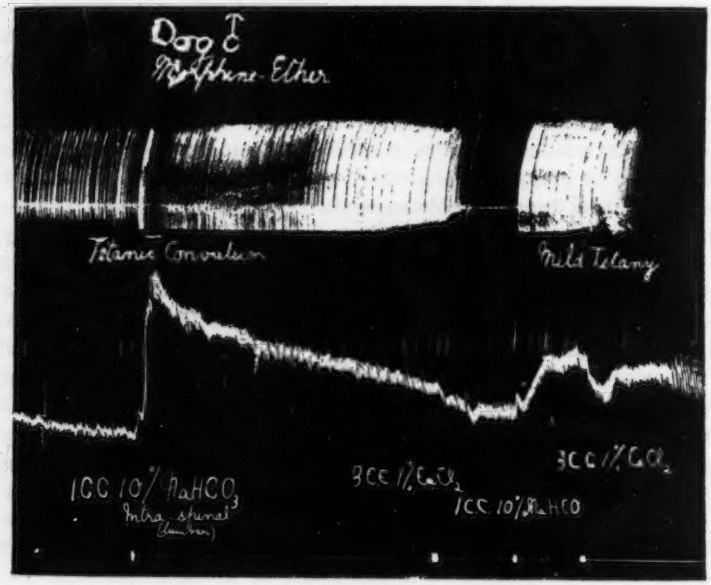


Fig. 1. Tetanic convulsions following intra-spinal injection of $NaHCO_3$. Effect antagonized by $CaCl_2$. Time, 3-second intervals.

action of the latter upon the nerve cells of the medulla. This was demonstrated in the following manner. A cannula was placed in the left carotid artery and connected with a mercury manometer. The right vertebral and carotid arteries were then dissected free and $NaHCO_3$ was injected at different periods into both of these vessels. In other instances injections were made into the central end of the ligated carotid artery. It was found that intra-arterial injection of this type resulted at once in stimulation of the medullary centers.

The immediate effect of simultaneous stimulation of the respiratory, vasomotor and cardio-inhibitory centers by $NaHCO_3$ varied in different animals. The first effect was in some cases complete cardiac inhibition marked by a great fall in blood pressure (fig. 2). This however passed off in a few seconds, and the blood pressure rose to a higher point than it was formerly. That the cardiac inhibition just mentioned was due to strong stimulation of the cardio-inhibitory center was shown

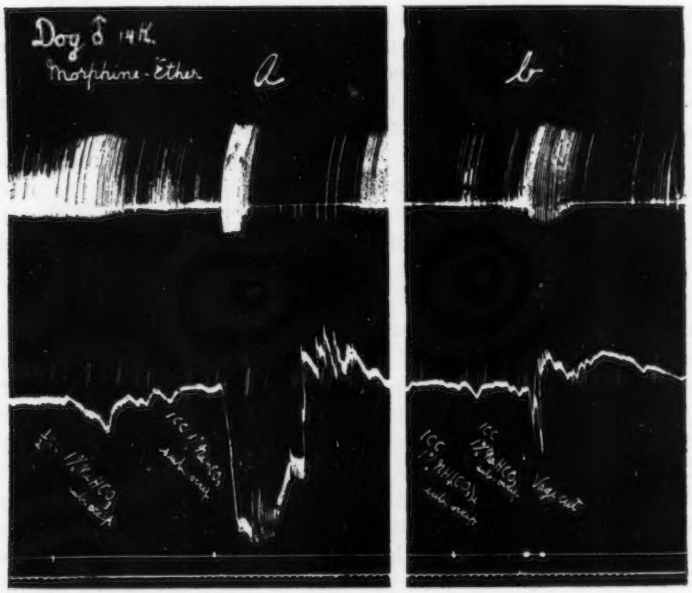


Fig. 2. *a*. Vagus inhibition following injection of $NaHCO_3$ into cisterna magna. *b*. Same after 4 minutes. Effect of $(NH_4)_2CO_3$ and of $NaHCO_3$. Vagi cut immediately after injection of $NaHCO_3$. Time, 3-second intervals.

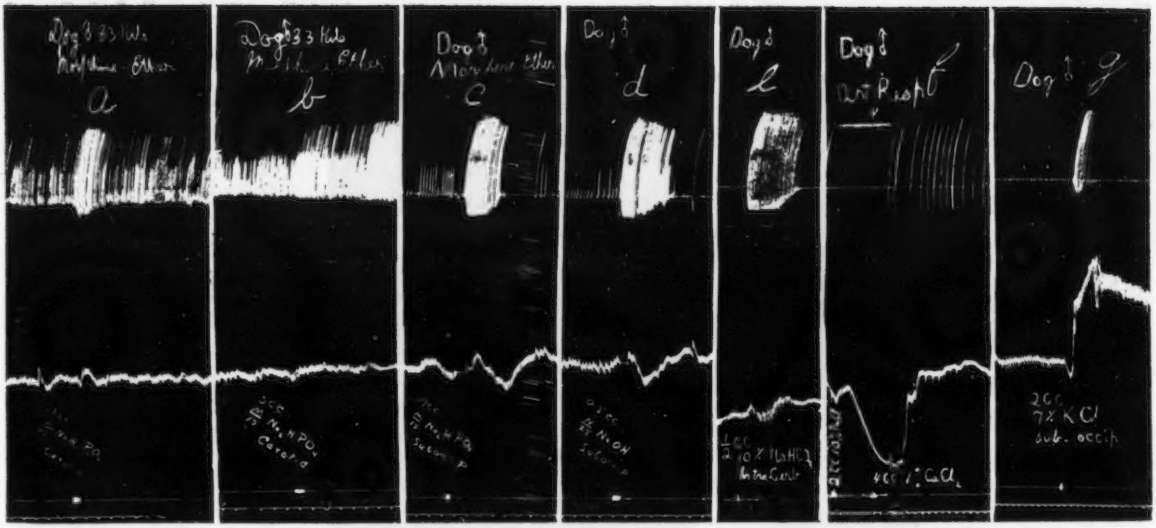
by cutting both vagi. When the vagi were cut sub-arachnoid injection of $NaHCO_3$ resulted in an immediate and extensive rise in blood pressure. As has been stated previously, slight tetany was obtained in dogs on two occasions after large intra-venous injections of $NaHCO_3$; this observation was the exception, however, and not the rule. It has also been demonstrated that occasionally one may obtain increased lung ventilation in dogs under ether anesthesia following intra-venous in-

jection of sodium bicarbonate. Under morphine-ether, however, the usual effect of $NaHCO_3$ given by intra-venous injection is a decrease in respiratory activity.

Sub-arachnoid injections of Locke's solution. It was found that sub-arachnoid and intra-cerebral injection of normal Locke's solution was without marked effect, while injection of concentrated Locke's solution caused definite stimulation of the medullary centers.

Sub-arachnoid injections of distilled water. Sub-arachnoid injection of 2 cc. of distilled water in the lumbar region of an unanesthetised rabbit caused after a latent period of one and one-half minute a tetanic convulsion which lasted for a period of seven minutes. Artificial respiration was performed during this period. Spontaneous respirations were resumed with the relaxation of the muscular spasm and an increase both in the amplitude and the rate of the respiratory movements was noted (protocol 4). During the next twenty minutes the tetany gradually subsided, the animal gaining control of the muscles of the anterior half of the body first, while at the end of half an hour it was able to run about. Sub-arachnoid injection of distilled water into the lumbar region of anesthetised dogs was followed by a rise in blood pressure, greatly increased lung ventilation and mild tetany of the muscles of the posterior half of the body. Injection of distilled water into the sub-cerebellar cistern caused stimulation of the medullary centers. Rapid injection of distilled water into the vertebral or carotid artery caused slight increase in blood pressure and very mild stimulation of the respiratory center (fig. 4).

Sub-arachnoid injections of sodium chloride. Hypertonic solutions of NaCl injected into the sub-arachnoid space of the lumbar region of rabbits produced practically the same type of effect as $NaHCO_3$ or distilled water (protocol 5). Injection of hypertonic NaCl solution into the lumbar region of the spinal canal of anesthetised dogs caused a mild degree of tetany, increased respiratory movements and a rise in blood pressure. The tetany was antagonized by $CaCl_2$; Weed and Wegeforth (2) have previously observed toxic effects during irrigation of the spinal canal with isotonic NaCl. Injections of hypertonic NaCl solution into the sub-cerebellar cistern resulted in strong stimulation of the medullary centers. If too great a quantity were injected these centers were paralyzed but could be again activated if $CaCl_2$ were injected at once (fig. 3). The injection of hypertonic NaCl into the vertebral or carotid artery caused slight stimulation of the medullary centers.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Sub-arachnoid injections of KCl. Hypertonic solutions of KCl injected into the sub-arachnoid space or into the carotid or vertebral artery were found to have the same effect as hypertonic solutions of NaCl, but to a much greater degree (fig. 3, *g*). $CaCl_2$ antagonized the action of KCl in much the same manner as it did NaCl.

Sub-arachnoid injections of $(NH_4)_2CO_3$, $MgSO_4$, Na_2HPO_4 *and* NaH_2PO_4. $(NH_4)_2CO_3$ was found to have a mild stimulating effect on

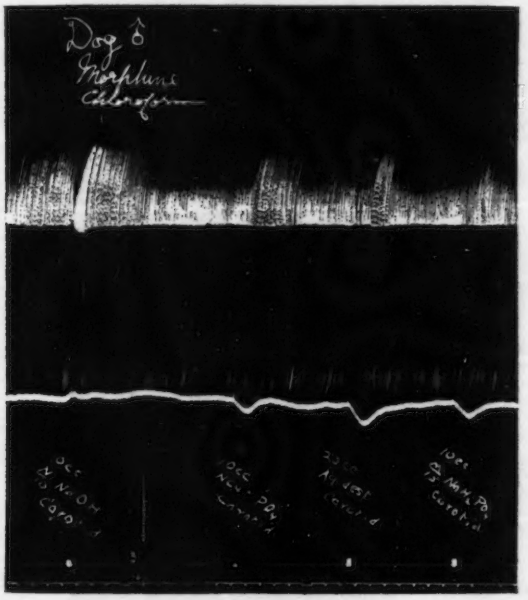

Fig. 4. Effect of injection into carotid artery of $\frac{N}{10}$ NaOH, $\frac{M}{15}$ neutral phosphate, distilled water and $\frac{M}{15}$ acid phosphate. Time, 10-second intervals.

the medulla (fig. 2), while $MgSO_4$, as has been well established, had a depressant action. Both basic and acid phosphates injected into the subcerebellar cistern caused increased lung ventilation while neutral phosphate had a slight stimulating action. Intra-arterial injection of both acid, basic and neutral phosphate solutions caused increased respiratory activity (figs. 3 *a*, and 4). Intra-spinal injection of basic phosphate produced tetany in the rabbit (protocol 6).

Sub-arachnoid injections of NaOH. One cubic centimeter of $\frac{N}{10}$ NaOH injected into the lumbar sub-arachnoid space of a rabbit caused mild tetany in all muscles of the body which was followed by a temporary paralysis of the muscles of the lower extremities (protocol 7). Three cubic centimeters N. sodium hydroxide injected into the lumbar region of the spinal canal of an 8-kilo dog under the influence of morphine caused increased tonus of all muscles. Two cubic centimeters of 10 per cent $NaHCO_3$ injected into the same region after an interval of twenty minutes caused intensive tetanic spasms of the whole musculature. Three cubic centimeters of $\frac{N}{25}$ NaOH injected into the lumbar region of a dog under morphine-ether anesthesia produced very little effect while 1 cc. of $\frac{N}{10}$ NaOH injected into the sub-cerebellar region of another animal caused a decrease in respiratory movement and a slight fall in blood pressure. One-half cubic centimeter of $\frac{N}{25}$ NaOH placed in the sub-cerebellar cistern caused in another instance decreased lung ventilation and stimulation of the cardio-inhibitory and vasomotor centers. Injection of 10 cc. of $\frac{N}{25}$ NaOH into the carotid artery of one animal caused definite stimulation of the respiratory center (fig. 4).

Irrigation of the spinal canal. Irrigation of the spinal canal with isotonic Ringer-Locke's produced little effect, while irrigation of the sub-arachnoid spaces with Ringer-Locke's containing 0.3 per cent $NaHCO_3$ produced intense hyperpnoea. It was found when the cerebrospinal fluid was washed out from the sub-arachnoid spaces and replaced by isotonic saline that, after a period of half an hour had elapsed, it was practically impossible to secure fluid from the sub-cerebellar cistern. This was attributed to the inability of the practically protein-free fluid to resist absorption from the sub-arachnoid area.

The protocols which follow indicate in greater detail the manner in which some of the experiments referred to above have been carried out.

PROTOCOLS

1. Rabbit ♂, weight 2 kilos

3:55 p.m. Cocaine hydrochloride 0.5 grain subcutaneous and intra-muscular over the fourth lumbar vertebra.

4:00 p.m. Injection of 2 cc. 10 per cent $NaHCO_3$ in distilled water into the sub-arachnoid space of the lumbar region.

4:01 p.m. Tetanic convulsion onset of which caused the animal to turn a complete somersault.

4:01 to 4:30 p.m. Gradual diminution of the tetany with hyperpnoea throughout.
4:45 p.m. Animal able to run around.
Animal died the following day.

2. Dog ♀ 6.5 kilos

3:45 p.m. 1 gr. morphine subcutaneous.
4:10 p.m. 0.5 gr. of cocaine subcutaneous and intra-muscular over fourth lumbar vertebra.
4:17 p.m. 4 cc. of 5 per cent $NaHCO_3$ injected into the lumbar sub-arachnoid space.
4:17½ p.m. Tetanic spasm of the entire body but most marked in the posterior half.
4:30 p.m. Tetany practically gone.
4:50 p.m. 2 cc. of 1 per cent $CaCl_2$ injected into the lumbar sub-arachnoid space.
5:00 p.m. 5 cc. of 5 per cent $NaHCO_3$ injected without effect.

3. Dog ♂, 40 kilos

10:25 a.m. 4 gr. morphine subcutaneous.
11:00 a.m. Ether anesthesia.
11:15 a.m. Tracheotomy and cannula in left carotid connected for recording blood pressure.
11:40 a.m. Lumbar puncture. Clear fluid from needle.
11:45 a.m. 1 cc. of 10 per cent $NaHCO_3$ injected through needle in lumbar sub-arachnoid space.
Tetany confined to posterior half of body developed at once, rate and depth of respirations increased, and increase in blood pressure from 120 mm. to 200 mm. Hg.
11:55 a.m. 1 cc. of $CaCl_2$ by lumbar needle.
11:58 a.m. 1 cc. of 10 per cent $NaHCO_3$ by lumbar needle. No tetany. Hyperpnoea and rise in blood pressure from 130 mm. to 165 mm. Hg.

4. Rabbit ♂, 2 kilos

12:00 noon. 0.5 gr. cocaine hydrochloride sub-cutaneous and intra-muscular over fourth lumbar vertebra.
12:05 p.m. Lumbar puncture.
12:06 p.m. 2 cc. distilled water slowly injected into spinal canal.
12:07½ p.m. Intense tetanic spasm of all muscles.
12:10 to 12:13 p.m. Artificial respiration.
12:13 to 12:45 p.m. Tetany diminished gradually.
12:45 p.m. Animal has control of fore-limbs, head and shoulders.
12:55 p.m. Animal able to run about.
Next day. Completely recovered.

5. Rabbit ♂, 1.5 kilo

4:00 p.m. 0.5 gr. cocaine hydrochloride sub-cutaneous and intra-muscular over fourth lumbar vertebra.
4:05 p.m. Lumbar puncture.
4:07 p.m. 2 cc. of 10 per cent NaCl intra-spinal.
4:08 p.m. Tetany of whole musculature.
4:13 p.m. Tetany considerably diminished.
4:30 p.m. Animal running about.
Next day. Animal normal.

6. Rabbit ♂, 2 kilos

4:00 p.m. 0.5 gr. cocaine hydrochloride sub-cutaneous and intra-muscular over fourth lumbar vertebra.
4:05 p.m. 0.5 cc. $\frac{M}{15}$ Na_2HPO_4 intra-thecal.
4:05½ p.m. Tetanic spasm most marked in posterior extremities.
4:10 p.m. Tetany practically gone.
4:15 p.m. Animal running about.
4:20 p.m. 0.5 cc. of NaH_2PO_4 intra-thecal; no effect.

7. Rabbit ♂, 2 kilos

4:00 p.m. 0.5 gr. cocaine hydrochloride sub-cutaneous and intra-muscular over the fourth lumbar vertebra.
4:02 p.m. Lumbar puncture.
4:05 p.m. 0.5 cc. of $\frac{N}{10}$ NaOH intraspinal.
4:05½ p.m. Mild tetany of whole musculature.
4:15 p.m. Tetany practically gone but animal has no control of lower extremities.
5:00 p.m. Only partial control of hind legs.
Next day: Slight paralysis of posterior extremities.
Five days later: Complete recovery.

DISCUSSION

It was thought at first that the tetany following intra-spinal injection of $NaHCO_3$ was due in part to the change in the C_H of the nerve cells as a result of increased concentration of bicarbonate and in part to the specific action of the HCO_3 ion. When, however, further work disclosed the fact that similar effects could be elicited by intra-spinal injections of such substances as distilled water, NaCl, KCl and NaOH, it became apparent that the factors of H and HCO_3 ion concentration were probably of secondary importance. The change in concentration of Ca ion within the nerve cell either relatively or absolutely in relation to the

concentration of Na and K ions would seem to be the direct cause of the symptoms manifested in these experiments. As the effect of $NaHCO_3$ is relatively greater than that of the other electrolytes studied, especially of NaOH, it is possible that the bicarbonate ion as such may have a specific action. This would concur with the opinion of Macleod (7) that the respiratory center is sensitive to the HCO_3 ion. It is assumed, therefore, that change in the relative as well as absolute concentrations of the various kations is the chief underlying cause for the various phenomena noted in the experiments which have been quoted. Disturbance of the concentration of the Ca ion is, however, the chief factor. As physiologically balanced saline solutions are practically without effect while distilled water and solutions of Ca-free electrolytes in various concentrations produce such marked symptoms, it is evident that in all these latter instances the concentration of the Ca within the nerve cell must have been reduced as a result of the diffusion of the same out of the cell due to the difference in concentration established as a result of the injection. The administration of weak alkaline solutions such as NaOH, $NaHCO_3$ and Na_2HPO_4 would cause as well an actual fixation of a certain amount of Ca within the tissue, the concentration of Ca ion thereby being appreciably diminished.

The fact that sub-arachnoid injection of sodium bicarbonate caused effects which in magnitude are out of all proportion to the somewhat similar effects produced by NaOH would tend to show that the HCO_3 ion or the $NaHCO_3$ molecule has a specific stimulatory action. Large amounts of $NaHCO_3$ intra-spinal are definitely toxic, and result in paralysis of the motor nerve cells. It is also true that paralysis of the respiratory center results after large injections of distilled water or almost any neutral electrolyte. It is possible that the phosphate ion may also have a specific stimulatory action on the medulla as both the acid and basic salts produce somewhat similar effects on the medullary centers. Intra-spinal injections into the lumbar region, however, disclosed the fact that tetany is readily produced by the basic but not by the acid salt. This, taken in conjunction with the medullary stimulation by acid, basic and neutral phosphate solutions suggests a certain amount of specific action of the phosphate ion on the medulla.

The tetany which is produced by lumbar sub-arachnoid injection of $NaHCO_3$, distilled water, hypertonic solutions of NaCl and other electrolytes, is due in the main to direct stimulation of the motor cells in the anterior horn. One cannot exclude, however, the possibility of the stimulation of the anterior horn cells as a result of irritation of the

afferent fibers in the posterior roots or the intercalated neurones in the spinal reflex arcs. The stimulation of the respiratory and vasomotor center must under the above circumstances be largely reflex as the effect is manifested before the injected fluid can possibly reach the medullary centers. When, however, one makes the injection into the sub-cerebellar cistern there is, no doubt, direct stimulation of the nerve cells in the medulla. The fact that cardiac inhibition through the vagi is produced much more readily by sub-cerebellar injection than by lumbar injection is very significant in this respect. The cardio-inhibitory center is not stimulated reflexly to the same degree as are the vasomotor and respiratory centers, while direct stimulation of the medullary centers results in a very definite cardiac inhibition, which is manifested by a marked fall in blood pressure. This effect then gives way and the blood pressure rises due to general vasoconstriction.

The experiments which are quoted in this paper demonstrate very clearly that substances in solution administered by the sub-arachnoid channel act in a very short space of time directly on the nerve cells of the brain and cord. While the effects following sub-arachnoid injections are, for obvious reasons, much more pronounced than those following injections into the carotid or vertebral artery, yet the similarity of the symptoms manifested after arterial and sub-cerebellar injection of $NaHCO_3$, for example, are very significant. The very definite stimulation of the respiratory and vasomotor centers following intra-arterial injection of a potentially strong alkali like 10 per cent $NaHCO_3$ is positive proof of the sensitivity of these centers to the HCO_3 ion irrespective of blood C_H. The decrease in lung ventilation which follows, as a rule, slow and continuous intra-venous injections of $NaHCO_3$, is probably due to the lowering of the hydrogen ion concentration of the blood, without there being a concomitant increase of sufficient magnitude in the bicarbonate ion to neutralize or to overcome the effect of the latter. Definite stimulation of the respiratory center can, however, be occasionally obtained during intra-venous injection of $NaHCO_3$. It is of interest in this respect that such an effect is more likely to be manifested by an animal under ether rather than under morphine-ether anesthesia. When massive doses of $NaHCO_3$ are given comparatively rapidly by intra-venous injection, it is possible to obtain sudden collapse of the heart without paralysis of the respiratory center. In one instance spontaneous respiratory movements continued for two minutes after the heart had failed completely.

While it is possible to obtain direct action of a substance on the nerve cells by sub-arachnoid injection, the passage of such substance into the general circulation may take place very slowly or in some instances it may not be removed from the spinal canal at all (8).

The demonstration of Wilson (9) that parathyroid tetany is associated with an alkalosis of the blood, and the findings of MacCallum, Kellogg and Voegtlin (10) that symptoms like those of tetany can be induced by deficiency of calcium and symptoms following parathyroidectomy relieved by calcium administration would be amplified by the experiments herein recorded.

CONCLUSIONS

It would seem logical to conclude that the tonus of effector nerve cells and probably of all nerve cells is regulated not only by the C_H of the blood and spinal fluid but also by the maintenance in them of a definite equilibrium between the various ions. Of these latter the Ca ion is preëminent. Decrease in Ca ion leads to increased tonus, and increase in the Ca ion to decreased tonus. Slight increase in the concentration of either Na or K is marked by definite stimulation of the medullary centers while the stimulatory effect of the bicarbonate ion is very definite. The importance of Na, K and Ca ions as regards regulation of the respiratory center has been suggested by Howell (11).

It would seem probable that one of the underlying causes of tetany, as observed clinically, is a disturbance in the kation equilibrium within the nerve cells of the brain and cord.

Results, such as are herein reported, serve to emphasize the importance of definite ions of electrolytes in definite concentration within the living cell, a principle which has never been lost sight of since the pioneer experiments of Sidney Ringer (12) were published.

In conclusion I wish to acknowledge the assistance of Dr. P. L. Backus in the experimental work reported in this paper.

SUMMARY

1. Sub-arachnoid injection on the lumbar region of $NaHCO_3$, NaCl and distilled water is followed by violent tetany and definite stimulation of the medullary centers.

2. $CaCl_2$ administered by intra-spinal injection antagonizes tetany so produced.

3. Injections of $NaHCO_3$, NaCl and KCl into the sub-cerebellar cistern cause marked stimulation of the respiratory, vasomotor and cardio-inhibitory centers. $CaCl_2$ antagonizes this effect to a limited extent.

4. Injection into the carotid or vertebral artery of $NaHCO_3$, NaCl or KCl caused definite stimulation of the respiratory and vasomotor centers.

5. Intra-venous injection of $NaHCO_3$ may cause increased lung ventilation.

6. It is held that the bicarbonate ion has a specific stimulatory effect upon the nerve cells of the medulla and the motor cells of the anterior horn.

7. Disturbance in the concentration of the various kations particularly of the Ca ion within the nervous tissue produces most marked effects.

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THE INFLUENCE OF INTERNAL SECRETIONS ON BLOOD PRESSURE AND THE FORMATION OF BILE

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In connection with work previously reported (1) on the influence of internal secretions on the formation of bile, a study of the blood pressure was made. The object of this was twofold: to observe the effect on blood pressure of the particular gland substance being studied, and to determine what relation, if any, existed between blood pressure changes and the amount of bile secreted after a gland substance had been administered intravenously. The following gland substances were employed: mammary, orchic, ovarian, pancreatic, splenic, thymic and thyroid. These were all obtained from Armour & Company. To this list was added solution of adrenalin chloride prepared by Parke, Davis & Company, and secretin prepared by a method described in connection with other experiments (2). These were all given by intravenous injection. Nearly all of the experiments were performed on dogs but occasionally cats were used. A description of the technique will be found in the article to which reference has been made (1) and the numbers of experiments mentioned in this contribution correspond to the experiment numbers there given. All observations were made for arbitrary periods of twenty minutes each. The usual procedure was to record blood pressure and the number of drops of bile secreted during three twenty-minute periods, one immediately preceding the administration of the gland substance, a second commencing with the beginning of the injection, and a third immediately following the second. In a few cases the third record was not secured. The dose in every experiment, except those with adrenalin and secretin, was 10 mgm. of the gland substance per kilogram of body weight of the animal, dissolved in 100 cc. of physiological saline solution. This was warmed to 37°C. on a water bath and injected into the jugular vein by means of a burette. The dose of adrenalin was 0.1 cc. of a 1:1,000 solution of adrenalin chloride per kilogram of body weight, and the dose of secretin was 10 mgm. of a dried acid extract per kilogram.

Adrenalin. Five determinations were made with adrenalin. There was invariably an immediate rise of pressure which averaged 81.25 mm. of mercury. In the case of adrenalin the injections were made much more slowly than with the other substances studied and the maximum to which the pressure was allowed to rise was 220 mm. This high blood pressure, with slight fluctuations, was maintained for 5 minutes as an average and at the end of the 20-minute period the pressure had always fallen to or slightly below the original level.

The amount of bile secreted after the injection of adrenalin was always less than during the preceding 20 minutes and the striking feature was the marked decrease during the period of high blood pressure. For example, in experiment 13 the initial count was 140 drops distributed fairly uniformly throughout the period; during the first 5 minutes of the injection period 1 drop was secreted, in the next 5 minutes 3 drops, in the third 5 minutes 3 drops, and during the last 5 minutes 42 drops—a total of 49 drops for this 20-minute period.

The diminution produced by adrenalin in the amount of bile secreted may be explained by a decrease of both the arterial and venous inflow. As has been shown by Burton-Opitz (3), (4) adrenalin injected into the hepatic artery or portal vein exerts a local constricting action and diminishes the inflow and it is not improbable that even when the adrenalin is introduced through a vessel more distant from the liver the same action takes place.

Mammary. Mammary substance was used eight times and in all but one there was an initial rise in blood pressure followed by a fall and a slow return to normal. The average figures for these experiments are as follows: blood pressure at beginning of injection 109.2 mm.; maximum pressure 117.6 mm., reached 26.4 seconds after injection started; minimum pressure 95.4 mm., 71.4 seconds from beginning of injection; pressure 103.8 mm. at end of first 5 minutes, and 106.8 mm. at end of period. Experiment 46, which failed to show an initial rise in blood pressure, was similar in other respects to the experiments of the group. Ott and Scott (5) report a slight fall in blood pressure for a few seconds after intravenous injection of mammary substance but there is no record of the preliminary rise which occurred so uniformly in our experiments.

So far as bile production was concerned the effect of mammary gland substance was not uniform but it would seem to be a temporary decrease. In experiment 11 the falling off in the rate of secretion was most marked immediately after the injection. Twenty-three drops of

bile were secreted during the twenty-minute period following the injection as compared with an initial count of 46; 14 of the 23 drops came during the last half of the period whereas the original 46 drops had been quite uniformly distributed. Experiment 8 was similar; the initial count was 3 drops, but after the injection there was no secretion until the last 4 minutes of the period when 2 drops were recorded. On the other hand, experiments 16, 46 and 57 showed a progressive decrease in the formation of bile. The other experiments of the group showed no change or an increased production.

From these records it appears that not only is mammary substance inconstant in its effect on the secretion of bile but that there is no relation between changes in blood pressure and bile formation under its influence.

Orchic. Seven experiments were made with orchic substance. Four showed an initial rise of pressure followed by a fall slightly below normal, a gradual return to the normal level and the pressure at the end of the period approximately the same as at the beginning; two did not show an initial rise, but a slight fall and then a gradual rise above the original level persisting to the end of the period. In one, experiment 4, no blood pressure record was made. The average figures for the first group of experiments, viz., 18, 41, 42 and 55, are as follows: blood pressure at beginning of injection, 128 mm. of mercury; maximum pressure 147.2 mm., reached 62.5 seconds from beginning of injection; minimum pressure 124 mm. at 110.7 seconds after injection commenced; pressure at end of first 5 minutes, 134.7 mm., and at end of period, 126.5 mm. For the second group of orchic experiments, numbers 19 and 62, the initial pressure is 113 mm., followed by a minimum of 99 mm. in 20 seconds, a rise to 127 mm. in 125 seconds, 123 mm. at end of first 5 minutes and 132 mm. at the end of the period.

The reported observations of the effect of the intravenous injection of testicular extract on blood pressure vary somewhat but agree in general that the pressure is lowered. In 1901 Dixon (6) described an immediate and considerable transient fall of blood pressure accompanied by cardiac slowing following the injection of orchitic extract. Vincent and Sheen (7) and Miller and Miller (8) note the production of a fall in pressure after testicular extract. Vincent (9) and Ott and Scott (5) state that the fall in pressure is slight and lasts for a few seconds only. Wheelon (10) records a slight fall in blood pressure after castration. Bingel and Strauss (11) were unable to produce a change in pressure by the administration of extract of testis.

Orchic gland substance caused a decrease in the amount of bile formed in five of the seven experiments and in four of these the effect was progressive, a further decrease occurring in the second 20-minute period after the injection. One interesting feature presents itself in an analysis of these records: there was a primary decrease during the first 15 minutes following the injection, a rise during the next 15 minutes, and a second decline beginning about 30 minutes after the gland substance was administered. This second depression of secretory activity was more marked than the first.

A comparison of blood pressure changes with variations in the amount of bile secreted fails to show any uniformity. Of the four experiments showing a rise in blood pressure, then a fall and a gradual return to normal, three gave a decrease in bile formation and one, number 55, an increase of 108.33 per cent. In the two experiments where there was a fall of pressure followed by a rise to a point 18 mm. above the original height at the end of the period, the changes in pressure were accompanied by decreased production of bile in one, experiment 19, and increased production in the other, experiment 62.

Ovary. Ovarian substance was used five times, in four of which blood pressure records were made. Two cases, experiments 20 and 59, showed a rise of pressure shortly after the injection began, then a fall below normal, a second rise above the original level, and a gradual return to normal. Averages: initial pressure, 130 mm. of mercury; first maximum, 148.5 mm., 19.5 seconds after injection began; minimum 119.5 mm., 44 seconds from beginning; second maximum 137.5 mm. at end of first 3 minutes, and final pressure 132.5 mm. Two other experiments, 43 and 56, with ovarian substance failed to show changes similar to the above, the pressure remaining almost constant. In experiment 5 no blood pressure record was secured.

The effect of ovarian gland substance or extract of ovary on blood pressure has been reported by Ott and Scott (5), Vincent and Sheen (7), Miller and Miller (8), Vincent (9) and Gonalons (12). These investigators agree that the effect is a lowering of blood pressure which is usually slight and transient.

Ovarian substance invariably produced a decrease in the amount of bile formed. The average decrease was 44.59 per cent in the first period and 59.36 per cent in the second period after the injection.

Pancreas. This substance was employed in nine experiments and for six of these complete blood pressure records are available. The effects on blood pressure fall into two groups, one showing a prompt

and very marked fall in blood pressure, the other showing a preliminary slight rise followed by a fall. In all of these records there is a rather characteristic lowering of blood pressure which is still in evidence at the end of the period, but from which there is gradual recovery during the second 20-minute interval. Forty minutes after injection the blood pressure averaged 121 mm. as compared with 123.6 mm. immediately preceding the injection.

The first group consists of four experiments, 22, 38, 48, 50, and shows the following: initial pressure 123.2 mm.; fall began in 5.5 seconds and the minimum pressure, 60 mm., was reached 26.7 seconds from the beginning of the injection. At the end of 5 minutes the pressure was 86.5 mm., and at the end of the period, 95.7 mm.

The second group, experiments 14 and 52, gives these averages: initial pressure, 124 mm.; 17 seconds later pressure began to rise and reached a maximum of 131.5 mm. 19 seconds after the injection was started; then fell to 77.5 mm., 40 seconds from beginning; at end of first 5 minutes was 118.5 mm., and at end of period 107 mm.

With the exception of Popielski (13), who reports a marked and prolonged rise in blood pressure as the result of the injection of an acidulated watery extract of pancreas, investigators agree that injection of the substance of the pancreas or of saline extracts of pancreas causes a fall in blood pressure. Ott and Scott (5) state that the lowering of pressure is more marked than that obtained with ovary, testis, mammary, spleen and thymus.

Biliary secretion was decreased in every case in which pancreatic substance was administered. The reduction averaged 45.45 per cent in the first period and 44.28 per cent in the second period following the injection. The striking feature in this connection was the great reduction in secretory activity immediately after the injection. The counts made during the periods preceding the injections show the drops of bile falling at a fairly uniform rate. In the first 10 minutes subsequent to the introduction of the gland substance only 19 per cent of the total secretion for the period was obtained, the other 81 per cent occurring during the second half of the period. In the next 20-minute period, the second after the injection, the rate of secretion was more uniform but still only a little more than one-half the original.

Secretin. Seven experiments were carried out with secretin. The blood pressure records show an average pressure preceding the injection of 114.1 mm. of mercury; 20.2 seconds after the injection was begun pressure commenced to fall and reached a low point of 56.8 mm.

40 seconds from the starting point; at the end of 5 minutes the pressure was 110.5 mm., and at the end of the period, 118.4 mm. In two experiments, 26 and 54 of this series, there was a slight rise of pressure immediately following the injection. This increase above the original pressure averaged 15 mm. of mercury and in experiment 26 was succeeded by a drop similar to that which took place in the other experiments of the group; in no. 54 there was no abrupt fall in pressure but a gradual decline with the pressure at the end of the 20-minute period nearly the same as at the beginning.

The amount of bile produced was greatly increased in every case in which secretin was employed. This increase averaged 241.52 per cent for the first 20 minutes after the injection and 413.78 per cent for the second 20 minutes.

Bayliss and Starling (14) state, "Acid extracts of the mucous membrane (of the duodenum and jejunum) normally contain a body which causes a fall of blood pressure. This body is not secretin, and the latter may be prepared free from the depressor substance by acting on desquamated epithelial cells with acid." This has been confirmed by v. Fürth and Schwarz (15). Matsuo (16) also concludes that the depressor substance is separate from secretin, especially as acid injected into the duodenum, while producing copious pancreatic secretion, was followed by no change in blood pressure. He was, however, not able to obtain a secretin preparation which did not produce some fall in blood pressure, but the degree of the fall and the activity of the various preparations were not at all proportionate.

In our experiments it will be observed that while there was, as a rule, a fall in pressure immediately following the injection this had been recovered from in 5 minutes and from then on the pressure remained within a few millimeters of the original. At the same time the amount of bile was tremendously increased during the 40 minutes the experiments lasted.

Spleen. Injection of substance of the spleen was practically without effect on blood pressure. Seven trials were made and the average pressure prior to the injection was 104.4 mm.; 20 minutes after the injection it was 107.4 mm. Only two experiments showed any fluctuations that could be attributed to the injection. In experiment 21 the pressure rose from 128 mm. to 135 mm. in 20 seconds, then fell to 124 mm. 22 seconds later, rose to 136 mm. 63 seconds later, and declined gradually to 129 mm. at the end of the period. The initial pressure in experiment 61 was 130 mm. With the beginning of the injection

blood pressure commenced to fall and in 40 seconds had reached 124 mm.; from there it rose gradually to 140 mm. at the end of the period.

Ott and Scott (5) report a slight fall in blood pressure for a few seconds after the intravenous administration of splenic substance. Vincent and Sheen (7) and Vincent (9) noted the production of a transient fall in pressure by splenic extract. Oliver and Schäfer (17) state that spleen produces a preliminary fall of pressure followed by a gradual rise above normal and then a gradual return to normal. Bingel and Strauss (11) found inconstantly a temporary rise of pressure followed by a sharp fall. Miller and Miller (8) state that in their hands saline extracts of spleen invariably caused a rise in blood pressure, which was usually but not always followed by a slight fall below normal.

So far as the formation of bile after the introduction of splenic substance by vein is concerned we found no constant effect. In the first period after the injection four experiments gave a decrease, two an increase and one no change. Alterations in the rate of secretion during the second period after injection were also without harmony.

Thymus. Five experiments were carried out with thymic substance and no consistent effect on blood pressure noted. Experiment 39 showed a slight rise in pressure followed by a sharp fall, and a return to normal 6 minutes after the injection began.

Where any effect on blood pressure was produced by the injection intravenously of thymic gland substance, watery extracts or saline extracts of the thymus, observers are almost unanimous in reporting it as a decrease. Reference is made to Ott and Scott (5), Miller and Miller (8), Schäfer (18), Popper (19), Basch (20) and Lucien and Parisoot (21). Popielski (13) states that an acidulated watery extract of thymus caused a rise of blood pressure.

Reference to our previous paper (1) on this subject will show that we found the amount of bile secreted after the administration of thymic substance to be decreased. This effect was still in evidence in three out of four experiments in which a count was made for a second period of 20 minutes after the injection.

Thyroid. Substance of the thyroid gland was injected into nine dogs. In two of the experiments, 6 and 33, no record of blood pressure was obtained. The other seven all show an initial rise in pressure, a sharp fall, a second gradual rise and a fall to normal. Pressure during the second 20-minute period following the injection was practically the same as in the period preceding the injection. Average figures for these experiments follow: initial pressure 105.8 mm.; first maximum

119.4 mm., 19 seconds from beginning; minimum 86.5 mm., 43.4 seconds from beginning; second maximum 117.7 mm., 191.4 seconds from beginning, and pressure at end of period, 110.2 mm.

Other observers have usually found a fall in blood pressure to result from the administration of thyroid extract or thyroid gland substance. Oliver and Schäfer (17), Haskovec (22), Georgiewsky (23), Guinard and Martin (24), Fenyvessy (25), v. Cyon and Oswald (26), v. Fürth and Schwarz (27), all report a fall in pressure. Ott and Scott (5) used iodothyrin and obtained a marked fall with subsequent gradual rise above normal. Schäfer (18) observed a considerable fall when using thyroid extract. Vincent (9) usually noted a fall but occasionally a rise of pressure. Levy (30) records no appreciable alteration in blood pressure after intravenous injection of Kendall's crystalline thyroid iodine compound. A rise in pressure is reported by Popielski (13), Heinatz (28) and Livon (29).

So far as bile formation is concerned thyroid substance was without constant effect. Experiments 6, 28, 40 and 51 show an increase averaging 82.29 per cent in the first period after the injection. Experiments 12, 17, 33, 37 and 58 show a decrease averaging 33.85 per cent for the same period. Nevertheless, in spite of the wide variations in amount of bile secreted, the blood pressure ran a very similar course in all of these experiments.

DISCUSSION

While the foregoing experiments are too few in number to permit definite conclusions to be drawn, one thing seems certain, viz., that there is no constant relation between blood pressure and the amount of bile secreted. Adrenalin, it is true, consistently raised blood pressure and lowered bile formation; secretin, on the other hand, where it caused a change in blood pressure, produced a lower pressure and a great increase in the flow of bile. It might be urged that thyroid gland substance owes any action it exerts upon blood pressure and bile formation to the intervention of the adrenals and this cannot be entirely controverted by our experiments. That thyroid substance increases the output of adrenalin has been shown by Bückner (31), Rudinger, Falta and Eppinger (32) and Gley and Quinquaud (33). In our experiments, however, there was no constant relationship between blood pressure and bile production after the administration of thyroid gland substance. We did not find bile production regularly decreased when blood pressure rose or vice versa. With others of the gland substances

employed the blood pressure might be lowered and bile production decreased at the same time. The most striking example of this is in the series with pancreatic substance. Intravenous administration of substance of the pancreas caused lowering of the blood pressure and lessening of the output of bile. To a lesser extent thymic substance acted in the same manner. If we place in contrast with this the effect of secretin it would seem that we are not justified in concluding that the effect on bile formation is due to the alteration in blood pressure.

With the other gland substances employed, viz., mammary, orchic, ovarian and splenic, the results were inconstant. Orchic substance, for instance, caused a rise of blood pressure in some of our experiments and a drop in others, while the production of bile was definitely lowered. After the administration of substance of the ovary the blood pressure showed oscillations, or waves of higher and lower pressure. The pressure at first rose above the original level, then fell below the initial, rose again and returned to the original. Biliary secretion was lowered in every case and an examination of the individual records of these experiments does not show any synchronism between changes in blood pressure and the rate at which the bile was secreted.

CONCLUSIONS

1. As a result of the experiments set forth in this paper we feel inclined to believe that some at least of the endocrine organs exert a specific influence on the secretory activity of the hepatic cells leading to the production of bile.

2. The output of bile in the dog is increased by the administration of secretin.

3. The output of bile in the dog is decreased by the administration of adrenalin, and by mammary, orchic, ovarian, pancreatic and thymic gland substances.

4. The amount of bile secreted is not affected in a constant or definite manner by the substance of the spleen and thyroid gland.

5. Blood pressure is raised by adrenalin.

6. Blood pressure is lowered by pancreatic substance and the secretin preparation employed.

7. A fall of blood pressure, ordinarily preceded by a slight rise, is caused by orchic and mammary gland substances.

8. Oscillations of blood pressure are caused by ovarian and thyroid gland substances.

9. Blood pressure is not usually affected by splenic and thymic gland substances.

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GASTRIN STUDIES

V. Chemical Studies on Gastrin Bodies

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As a result of numerous physiological studies with extracts from duodenal and gastric mucous membranes and from many other tissues investigators, at present, are divided into two schools. One school considers pancreatic secretin on the one hand and gastrin or gastric secretin on the other hand to be different chemical substances and to act as distinctly specific secretagogues. The other school considers the substances to be identical, that they are widely distributed "vasodilatins" and that they do not act as specific secretagogues. The former view is generally accepted in our modern textbooks and this is especially true as to pancreatic secretin. In a series of previous papers (1) we referred to the literature on gastric secretin and we there reported more particularly on the physiological phase of the work. Although much of the evidence there presented together with the investigations published by others leads us to believe that very likely secretin and gastrin are different substances; nevertheless, we have not been able to convince ourselves as to the correctness of either of the two views now held. In this paper we report observations which bear more particularly on the chemical phase of the work. It is evident that the isolation of these secretagogues in pure form will do more toward solving these important questions than biological studies with variously purified extracts.

METHODS

Animals. The studies reported here were controlled on dogs with Pawlow and chronic fistula stomachs. The same general procedure was followed as previously reported, that is, a collection of a one- or

two-hour spontaneous secretion before the injection of the solution and a one- or two-hour collection after the same. The volumes were noted and the free and total acidities titrated in the usual way. All the solutions used were slightly acid and were well sterilized before injecting intramuscularly.

Extraction. It may be recalled that Bayliss and Starling (2) attempted to meet Popielski's criticism as to the presence of "vasodilatins" in secretin solutions by extracing the dried material with absolute alcohol and discarding the absolute alcohol-soluble portion. In our earlier studies we also employed this method, but later we arrived at the conclusion that we were by no means warranted to assume that the absolute alcohol did not also remove some of the gastrin activity. The studies reported here are on material which in most cases has not been thus treated with absolute alcohol and which was prepared from filtered artificial gastric juice as prepared for the manufacture of pepsin. The filtered material as obtained from the factory was coagulated by heat, filtered, and the filtrate concentrated under diminished pressure in a bath of boiling water. The thin syrup thus obtained was neutralized to faint acidity and precipitated with five to six volumes of redistilled 95 per cent alcohol. After standing for several days the solution was filtered and the filtrate again concentrated under diminished pressure with complete removal of the alcohol. The solution left, if not distinctly acid, was made so and stored under toluol in glass stoppered cylinders. It retained its activity perfectly. This concentrated solution was used as the material for various attempts at further purification and for various studies as to stability.

PRECIPITATION STUDIES

Action of basic lead acetate. The addition of basic lead acetate in excess to this partly purified solution of gastrin does not precipitate the activity as is shown by a number of experiments in which both the precipitate and filtrate were freed from lead and tested physiologically. In each case very little or no activity was found in the precipitate and most of the original activity was recovered in the filtrate. As an illustration one of three equally reliable experiments is given below.

Dog III. Pawlow accessory stomach, January 14, 1915

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
	cc.	*per cent*	*per cent*
10:10 a.m. to 11:10 a.m.	1.1	0.09	0.12
11:10 a.m.	Injected 2 cc. Sb4B*		
12:10 p.m.	6.9	0.36	0.39
1:10 p.m.	1.4	0.36	0.40
1:10 p.m.	Injected 2 cc. Sb4A*		
2:10 p.m.	0.6	0.26	0.31
2:20 p.m.	Injected 2 cc. Sb4B*		
3:20 p.m.	2.20	0.27	0.32
4:20 p.m.	1.8	0.36	0.41

* A crude extract, Sb4, was precipitated with an excess of basic lead acetate, filtered and washed. The precipitate and filtrate were decomposed to remove the lead and concentrated under diminished pressure. Both solutions were then made up to the same volume so that 2.5 cc. were equivalent to 1.5 cc. of the original Sb4. Sb4B representsthe filtrate and Sb4A the lead acetate precipitate. This step in purification was frequently used in many of the other studies referred to below.

Precipitation by picric and picrolonic acids. Many attempts were made to precipitate the activity by picric acid in various acidities and concentrations, but in every case the activity was recovered in the filtrates. Picrolonic acid also yielded inactive precipitates. It is of particular interest to note, however, that when histamine hydrochloride is added to the same gastrin solution one obtains an active precipitate which undoubtedly is the histamine picrolonate. In these experiments the precipitate obtained was in each case suspended in dilute hydrochloric acid and shaken with benzol or amyl alcohol to completely remove the picric or picrolonic acid. The aqueous hydrochloric acid solution was then concentrated under diminished pressure and rediluted to the original volume. The filtrates from the precipitates were decomposed in the same way and also made up to the original volume after concentrating under diminished pressure. The results below are characteristic and were duplicated on other animals.

Dog III. Pawlow accessory stomach, January 22, 1916

TIME	JUICE	HYDROCHLORIC ACID		REMARKS
		Free	Total	
	cc.	*per cent*	*per cent*	
1st hour.....	0.7	0	Trace	Slime
Injected 1 cc. S1A (an alcoholic extract of dried crude gastrin)				
1st hour after..	7.5	0.36	0.46	
2nd hour after..	3.1	0.46	0.50	
Same dog on January 27, 1916				
12:30–1:30 p.m..	1.0	0	0.05	Mucin
1:30 p.m.....	Injected picrate precipitate from 1 cc. S1A			
1:30–2:30 p.m..	1.1	0	Trace	
2:30 p.m.....	Injected picrate filtrate from 1 cc. S1A			
2:30–3:00 p.m..	5.4	0.24	0.26	
3:00–3:15 p.m..	1.8	0.43	0.45	
3:15–3:30 p.m..	0.8	0.46	0.49	
3:30–4:00 p.m..	0.7	0.44	0.47	

If to 10 cc. of the same S1A solution we added 10 mgms. of histamine hydrochloride and then proceeded with the precipitation by picric acid in the same way and examined the precipitate and filtrate for activity, the following results were obtained on the same dog.

TIME	JUICE	HYDROCHLORIC ACID		REMARKS
		Free	Total	
	cc.	*per cent*	*per cent*	
1st hour.....	0.6	0	Trace	Mucin
Injected 1 cc. of the concentrated filtrate=1 cc. S1A				
1st hour after...	7.8	0.38	0.41	
2nd hour after..	5.9	0.50	0.54	
3rd hour after..	2.3	0.46	0.51	
Injected 1 cc. from precipitate=1 cc. S1A plus 1 mgm. histamine hydrochloride				
1st hour after...	0.7	0.27	0.28	
2nd hour after..	1.8	0.09	0.12	

The results indicate a very poor precipitation of the added histamine by the picric acid.

With picrolonic acid we have as stated before, not been able to obtain an active picrolonate precipitate from various gastrin solutions, but when we add histamine to the same solution and then precipitate in the same way with picrolonic acid we not only obtain the histamine in the precipitate but also recover the gastrin activity otherwise, just as before, in the filtrate. In conducting this experiment we first showed that we could quantitatively recover 0.010 gram histamine hydrochloride from 10 cc. water by precipitating with 50 cc. saturated aqueous picrolonic acid solution. This was shown chemically by the diazo test. Next the same amount of histamine hydrochloride was added to 10 cc. of S1A gastrin solution and precipitated in the same way by picrolonic acid. The precipitate and filtrate were decomposed in the usual way and after concentration made up to the original volume. The physiological response was as indicated in the protocol below.

Dog III. Pawlow accessory stomach, February 9, 1916

TIME	JUICE	HYDROCHLORIC ACID		REMARKS
		Free	Total	
	cc.	*per cent*	*per cent*	
1 hour.....	0.3	0.00	Trace	
½ hour.....	0.2	0.00	Trace	
Injected of the picrolonate filtrate=1 cc. S1A				
1st hour after...	5.8	0.37	0.43	
2nd hour after..	1.5	0.47	0.52	
Injected of the picrolonate precipitate=1 cc. S1A plus 1 mgm. histamine hydrochloride				
1st hour after...	5.5	0.37	0.41	
2nd hour after..	0.3	Present		

On comparing the results here with those obtained with 1 cc. of the original S1A as given in the table for January 22, we find that the response is of the same order for the picrolonate filtrate as for the original S1A. This together with the inability to precipitate gastrin by picrolonic acid shows that the added histamine was quite completely precipitated by the precipitant. This was also verified by the diazo test applied on the precipitate and filtrate. These studies certainly indicate that gastrin and histamine are not identical chemically although they both act as good secretagogues.

Precipitation by mercuric chloride in acid solution. After the removal of considerable foreign material by basic lead acetate and removal of the lead from the filtrate, the acid filtrate when treated with a fair excess of cold saturated aqueous mercuric chloride yields a precipitate possessing considerable activity. A second mercury precipitate could be obtained by adding an alcoholic saturated mercuric chloride solution, but both this precipitate as well as the filtrate therefrom possessed very little or no activity. There appears to be a distinct loss of activity in this mercury treatment. The protocol below illustrates the character of the findings.

Dog III. Pawlow accessory stomach, January 29, 1915

TIME	JUICE	HYDROCHLORIC ACID		REMARKS
		Free	Total	
	cc.	per cent	per cent	
1st hour.....	0.1	0	0	Mucus
10:50 a.m....	Injected 3 cc. Sb5D3*			
11:50 a.m....	0.1	Trace		Mucus
11:50 a.m....	Injected 1.5 cc. Sb5D2*			
12:50 p.m....	0.1	Trace		Mucus
12:50 p.m....	Injected 1.5 cc. Sb5D1*			
1:50 p.m....	3.5	0.38	0.41	
2:50 p.m....	0.9	0.38	0.47	

* The material started with Sb5B had been treated with basic lead acetate. The first mercury precipitate, Sb5D1, after decomposition and removal of the mercury was concentrated under diminished pressure and made up to the same volume as the original Sb5B started with. The second mercury precipitate, Sb5D2, was likewise freed from mercury and after concentration made up to the same volume as the original Sb5B started with. The filtrate from the second mercury precipitate, Sb5D3, similarly treated, was made up to twice the volume of the original Sb5B.

It is to be noted that nine days previously an injection of 1 cc. Sb5B in the same dog caused a secretion of 4.0 and 1.5 cc. gastric juice during the first and second hours following the injection with acidities of 0.46 and 0.50 per cent respectively. In other words, a dose of two-thirds the size caused a better secretion than any of the fractions above. Similar experiments on other dogs and repetitions of the same experiment on other extracts confirmed these findings.

Purine base precipitation. To 10 cc. of a solution which had been carried through the treatment with basic lead acetate and through the mercuric chloride precipitation in acid solution, we added sodium hydrate until the solution remained only very slightly acid. We now

added 2 grams sodium acetate, 2 cc. 35 per cent sodium bisulphite solution, 4 cc. 10 per cent copper sulphate solution and boiled for 3 minutes, filtered at once and washed with boiling water. Both the precipitate and filtrate were freed from copper by hydrogen sulphide, concentrated under diminished pressure and made up to definite volume. The precipitate contained no activity but the filtrate showed good secretagogue action.

Other precipitants. In part of the work, as shown below, it was discovered that the gastrin activity can be extracted from an alkaline solution by amyl alcohol and that it can be recovered therefrom by shaking with dilute hydrochloric acid in water. A solution was thus prepared from material which had been treated as above; that is, with lead acetate and mercuric chloride. The hydrochloride solution thus obtained was evaporated to dryness under diminished pressure, then redissolved in water and diluted to one-fourth the original volume. The original solution referred to was already a very active one. Of this solution 1 cc. portions were taken and treated on watch glasses as indicated below. The precipitates and filtrates were separated and freed from precipitating reagent as well as possible and were then tested for physiological activity and for color reaction by the diazo benzene sulphonic acid reagent.

REAGENT AND AMOUNT	IMMEDIATE RESULT	RESULT AFTER 20 HOURS
Saturated picrolonic acid, 2 cc.	Flocculent precipitate	Same
Alcoholic zinc chloride, 1 cc.	No change	No change
Alcoholic cadmium chloride, 1 cc.	No change	Slight precipitate
Iodine in potassium iodide, 3 cc.	Heavy brown to black precipitate	No change
Potassium mercuric iodide, saturated, 5 drops	Slight precipitate	No change
5 per cent platinic chloride, 1 cc.	No change	Reddish yellow precipitate
5 per cent gold chloride, 1 cc.	Heavy orange precipitate	No change
Saturated silver sulphate and $Ba(OH)_2$ until alkaline	Precipitate white and brown	Black precipitate

The results of the physiological and diazo tests are recorded below.

REAGENT	PRECIPITATE		FILTRATE	
	Diazo test	Physiological	Diazo	Physiological
Picrolonic acid	Negative	Negative	Positive	Positive
Cadmium chloride	Negative	Negative	Positive	Positive
I and KI	Negative	Negative	Positive	Negative
Platinic chloride	Negative	Negative	Positive	Positive (?)
Gold chloride	Negative	Negative	Positive	Positive
Ag_2SO_4 and $Ba(OH)_2$	Positive	Positive	Positive	Positive

The results again confirm the former findings as to picrolonic acid and also again suggest a similarity to imidazol derivatives in that the precipitation by silver in an alkaline solution is positive and in that the diazo reaction usually parallels the activity.

This precipitation by silver sulphate and barium hydrate was repeated later on larger quantities of material. It was found that not only was the activity left to a large extent in the filtrate but that there seems to be a loss of activity by this treatment.

STUDIES WITH IMMISCIBLE SOLVENTS

An active solution obtained by the mercuric chloride precipitation process was rendered alkaline to litmus by sodium hydroxide and then shaken with various immiscible solvents. These solvents were later shaken with dilute aqueous hydrochloric acid and the latter concentrated under diminished pressure, diluted to definite volume and tested physiologically. In every case a precipitate formed in the alkaline aqueous solution; this was filtered off and found physiologically inactive. The results on the immiscible solvents show that the gastrin is not soluble in carbon disulphide, chloroform, benzene, ether and ethyl acetate. The only immiscible solvent by means of which we were able to extract the activity from an alkaline solution is amyl alcohol. The amyl alcohol extraction was slow but clear-cut. We used Kahlbaum's purest grade amyl alcohol for the first trials and found that careful controls thereon showed no activity. Later we used the same alcohol as such or after recovery by shaking with alkaline solutions, removing the water by anhydrous potassium carbonate and fractionating by distillation. The discovery of the solubility in amyl alcohol was next applied on pure histamine solutions and there also we found that the free base can be removed from alkaline aqueous solutions by means of

this solvent.[1] This was shown by means of the diazo-benzene sulphonic acid reaction. An attempt was made to carry on a continuous Soxhlet extraction of a dry alkaline mixture of crude gastrin, but the amyl alcohol extract became very much discolored, gave indications of resin formation, and contained very little or no activity. Very likely the activity was lost in the prolonged extraction at the boiling temperature of amyl alcohol.

The similar solubilities of gastrin and histamine in amyl alcohol are suggestive, and indicate that the substances are of similar character. They do not appear to be identical because, as shown above, the gastrin solution obtained from an amyl alcohol extract does not yield a physiologically active precipitate with picrolonic acid as does histamine.

STABILITY STUDIES

Action of alkaline solutions. To 10 cc. of the same preparation as used for the immiscible solvent studies we added 5 cc. 10 per cent sodium hydroxide in excess beyond the neutral point and then evaporated to dryness on the water bath. The material was next redissolved in water, neutralized by dilute hydrochloric acid and diluted to definite volume. This solution was compared with one treated in the same way only the evaporation was conducted with the solution in a neutral condition. The protocol below gives the physiological responses from the material and shows the destructive action of alkali on gastrin.

Dog III. Pawlow accessory stomach, May 12, 1915

TIME	JUICE	HYDROCHLORIC ACID		REMARKS
		Free	Total	
	cc.	*per cent*	*per cent*	
9:34–10:34 a.m.	2.3	0.12	0.17	Some mucin
10:34–11:34 a.m.	2.0	Trace	0.09	Clear
11:34 a.m.–12:34 p.m.	1.0	Trace	0.05	
12:34 p.m.	Injected 1 cc. of the solution evaporated in neutral condition			
12:34–1:34 p.m.	11.0	0.41	0.45	Clear
1:34–2:34 p.m.	1.2	0.36	0.39	Clear
2:34 p.m.	Injected an equivalent amount evaporated in alkaline solutions*			
2:34–3:34 p.m.	5.7	0.35	0.36	Clear
3:34–4:34 p.m.	0.6	0.24	0.27	Clear

* Slight loss in injection, not enough to account for the difference in secretion.

[1] This property was later applied in the quantitative separation of histamine and histidine as recently published by Koessler and Hanke. Journ. Biol.Chem., xxxix, 521.

Action of acids. To a solution of gastrin obtained from the decomposition of mercuric chloride precipitate, there was added an equal volume of concentrated hydrochloric acid (sp. gr. 1.19) and this was boiled for twenty-four hours under a reflux condenser protected from oxygen by a pyrogallol seal. The acid was removed by evaporating to dryness under diminished pressure, redissolving the residue in water and again evaporating in the same way. The solution finally was diluted to the original volume. In the protocol below Sf is the original solution and SfA the acid treated material.

Dog III. Pawlow accessory stomach, March 25, 1915

TIME	JUICE	HYDROCHLORIC ACID		REMARKS
		Free	Total	
	cc.	*per cent*	*per cent*	
9:18–10:18 a.m.	1.9	Negative	Bare trace	Mucus
10:22a.m.	Injected 1 cc. Sf			
10:22–11:22 a.m.	7.4	0.29	0.32	
11:22–11:52 a.m.	0.8	0.27	0.33	
11:52 a.m.	Injected 1 cc. SfA=1 cc. Sf			
11:52 a.m.–12:52 p.m.	9.2	0.36	0.40	
12:52–1:22 p.m.	1.5	0.35	0.36	

In another experiment a similar preparation was boiled for forty-nine hours, without protection from oxygen, with 20 per cent hydrochloric acid. The physiological results confirm the findings given above. This remarkable stability in acid solution is further confirmed by our experience with gastrin solutions kept for months in a distinctly acid solution under a layer of toluol.

COLOR REACTIONS OF GASTRIN SOLUTIONS

It is to be noted that in the precipitation studies reported above one finds the diazo benzene sulphonic acid reaction positive in very nearly every case in which we found activity. This was found to be generally true in all our separations and, in fact, this color reaction was frequently used as a rapid test for detecting complete washing of precipitates to be discarded. The intensity of this color reaction always was found to parallel intensity of the physiological activity. It again suggests a similarity in the chemical character of histamine and gastrin. Other color reactions were carried out on variously purified gastrin

solutions with the conclusions given below. Negative reactions were obtained by ninhydrin, di-methyl amido benzaldehyde, glyoxylic acid and furfural condensation. The Millon reaction was very faintly positive with a heavy precipitate. Knoop's bromine reaction was positive as with histidine, and the biuret test was positive but developed slowly on a solution which had not been hydrolyzed with hydrochloric acid. After hydrolyzing for 49 hours the activity remained, but the biuret reaction now was negative while the bromine and diazo reactions remained positive. The xantho-proteic acid reaction was very faintly positive on the mercury precipitated gastrin preparation.

PHYSIOLOGICAL STUDIES WITH SUBSTANCES OF KNOWN COMPOSITION

It was thought that by following the secretion of gastric juice after the injection of various substances of possible biological importance, but of known composition, we might be able to throw some light on the probable chemical character of the active substance in the gastric mucous membrane. The results of these studies are given in the table below.

TABLE 1

SUBSTANCE	TOTAL DOSE	NUMBER OF EXPERIMENTS	PHYSIOLOGICAL RESULTS		
			Positive	Negative	Doubtful
	mgm.				
Triethylamine HCl	10.0	3		2	1
Tetraethylamine HCl	10.0	1		1	
Cystin	20.0	4	1	2	1
Tryptophane	20.0	4		4	
Creatine	7.5	4		4	
Creatinine	7.5	5		5	
Choline chloride	10.0–20.0	16	5	10	1
Histidine	10.0	2		2	
Methylimidazol HCl	10.0	1		1	
Hydroxymethylimidazol HCl	10.0	1		1	
Guanidine HCl	10.0	5		4	1
Glucosamine HCl	20.0	4		4	
Histamine	0.25–1.0	Numerous	All	0	0

Apparently the only substances which have caused a secretion are choline and histamine. However the response from choline is so uncertain and so slight as compared with gastrin solutions and other tissue extracts that the physiological action of these extracts cannot be attrib-

uted to the presence of choline as was at one time claimed by various investigators (3). It is of interest to note that we never were able to cause a secretion of gastric juice by feeding gastrin by mouth in two- to threefold doses, nor have we ever been able to detect gastrin activity in dog or human gastric juice even after concentrating under diminished pressure and injecting intramuscularly.

GENERAL DISCUSSION OF THE RESULTS

Our studies thus far indicate that the active substance in gastrin solutions obtained from hog gastric mucous membrane is a very stable substance in acid solutions, that it is not easily oxidized in acid or neutral solutions, but that it is destroyed appreciably by heating with strong bases in water solution. The active substance does not lose its activity by prolonged acid hydrolysis and thus appears to be a distinctly basic substance as shown by its precipitation from acid solution by mercuric chloride and by its behavior toward immiscible solvents in the alkaline and acid conditions. It is not precipitated in the purine base precipitation method. The constant diazo reaction also suggests a basic substance and is particularly interesting in that pilocarpine is also an imidazol derivative. All of these properties suggest a similarity to pancreatic secretin as reported by others, with the exception that pancreatic secretin is generally considered to be more labile, especially in an atmosphere of oxygen (4). The properties enumerated above, as well as the physiological action, further suggest the identity of gastrin and histamine, but thus far we have not been able to precipitate the activity of a gastrin solution by means of picric or picrolonic acid. The work of Abel and Kubota (5) also suggests that histamine is the active constituent in our extracts, but as their work was based mostly on physiological evidence the proof is by no means conclusive. Koessler and Hanke[2] have in fact not been able to confirm the work of Abel and Kubota in that they did not find histamine by careful chemical search in casein nor in the fresh ox hypophysis. The results of Abel and Kubota (5), Koessler and Hanke (6), Tomaszewski (7), Fawcett, Hackett and Rahe (8), as well as our own results indicate the very wide distribution of substances, probably of basic character and similar in physiological action, in many tissues and possibly in the proteins themselves. It is very interesting indeed that we find these substances in relatively high concentrations in the same organs in which ammonia is generally most

[2] Unpublished verbal communication.

prominent immediately after death. It may also be recalled that these substances do not act physiologically when given by mouth and that they appear to be absent from gastric juice. The substance or substances in question may later be found to be identical or very closely related, possibly peptamides of very closely related structures.

CONCLUSIONS

1. Our results suggest a striking similarity between histamine and gastrin.
2. Gastrin appears to be more stable than secretin is reported to be, although they are similar in many respects.
3. Gastrin has not been precipitated by picric or picrolonic acid.
4. Gastrin appears to be a basic imidazol derivative which can be extracted as the free base by amyl alcohol.
5. Thus far histamine and pilocarpine are the only known imidazol derivatives which stimulate the gastric mechanism to secretion.
6. Choline acts with uncertainty and then only faintly as compared with histamine or gastrin.

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ESSENTIALS IN MEASURING EPINEPHRIN OUTPUT WITH FURTHER OBSERVATIONS ON ITS RELATION TO THE RATE OF THE DENERVATED HEART

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The measurement of the rate at which epinephrin is given off from the adrenals under any given conditions necessarily involves the measurement of a mass and a time, since that rate is the quantity of epinephrin liberated per unit of time. It seems absurd to set this down in so many words. And the absurdity would strike every reader if our subject were the rate of output of blood from the left ventricle, the rate of output of carbon dioxide from muscles, the rate of hydrolysis of starch by amylase or the rate of excretion of urea by the kidney. But curiously enough, in the case of epinephrin some writers seem to assume that the mathematical conditions which govern the measurement of every velocity can be circumvented. And quantitative conclusions as to the influence of this or that factor upon the rate of epinephrin output have been confidently deduced from reactions which are not even specific qualitative reactions for epinephrin (such as the paradoxical dilatation of the pupil or acceleration of the denervated heart) and from observations which did not comprise a single measurement of the quantity of epinephrin given off per unit of time. Indirect qualitative methods may have value in corroborating the results of direct quantitative methods, but in case of conflict the presumption must be in favor of the latter. We have recently (1) had occasion to call attention to the bearing of this generally accepted principle upon certain discrepancies in the literature in regard to the influence of asphyxia upon the rate of epinephrin output.

The straightforward way of measuring the mass of epinephrin given off per unit of time is to collect blood from the adrenal veins for a known time and to assay its concentration in epinephrin. Various methods of obtaining the blood may be adopted depending on the kind of animal

and other conditions of the experiments. Thus, in good-sized dogs a cannula may be inserted into the lumbo-adrenal vein on one side extraperitoneally, and blood collected from one adrenal. In cats the most convenient method is to form a cava pocket. This is a method which has been employed by a number of investigators, including Hoskins and McClure[1] (2) who, in addition to the cava and the veins entering it which Biedl (3) tied off, ligated the abdominal aorta. We also prefer to tie the abdominal aorta and renal arteries, in order to prevent too much blood from stagnating in the hind end of the animal and the kidneys when the cava and renal veins have been ligated. The arteries are tied a little time before the veins, so that as much blood as possible may drain out of the occluded parts. There is no obvious reason why the exclusion of the hind legs and kidneys should affect the output or the nervous mechanism governing it. Indeed, the better blood pressure maintained after ligation of these vessels ought to favor rather than to hinder any reflex nervous effects elicited, e.g., by stimulation of the central end of sensory nerves or any excitatory effects upon nerve centers exerted by such conditions as asphyxia, since the conductivity of the reflex arcs and the excitability of the nerve centers must be better maintained when the blood flow is increased. The ordinary spinal reflexes are well obtained in the parts whose circulation has not been interfered with, and indeed in the hind end for some time after ligation of the vessels (see, e.g., protocol of cat 436).

When Doctor Cannon (4) attributes to the peculiar method adopted by us for collecting the blood our failure to corroborate his results, he does not explain how our method is unfavorable to the eliciting of the reaction. He attempts to show that the output of epinephrin measured by us in adrenal vein blood, collected from the cava and assayed on rabbit intestine (and uterus) segments, is an artificial phenomenon. He says "the effect of opening the abdominal cavity, clamping off the inferior cava and repeatedly manipulating the abdominal contents,

[1] The epinephrin output determined by these observers in 5 dogs by assay of adrenal blood, collected in the manner described, on rabbit intestine segments agrees well with our results. If the 1: 1,000,000 adrenalin solution, in terms of which the output is expressed, is assumed to contain about 75 per cent of base (it was probably not assayed) the average output would be 0.00019 mgm. per kgm. per minute. Our average for 17 dogs was 0.00022 mgm. per kgm. per minute. The average weight of our dogs was exactly half that of the dogs employed by Hoskins and McClure, and there is reason to believe that in the larger dogs the output of epinephrin per kilogram of body weight is somewhat less than in the smaller.

either in pressing blood out of the inferior cava or withdrawing it by syringe, must be examined." We do not press blood out of the inferior cava nor do we withdraw it by syringe, if that is a more serious insult to the animal, nor do we repeatedly manipulate the abdominal contents. We open the abdominal cavity, prepare the cava pocket and clamp it off when blood is to be collected from the cannula inserted in the lower end of the pocket. We have examined the influence of several of the factors mentioned upon the output and have not found any effect detectable by our methods (5), (6).

The alleged influence of opening the abdomen upon epinephrin secretion. Doctor Cannon does not state the result of his examination. All he says is that "fully twenty years ago Bayliss and Starling called attention to the profound effect which opening the abdominal cavity has on the intestines in causing them to become absolutely motionless. These well-established facts make an interesting commentary on the use of the cava pocket as a mode of obtaining evidence of normal or natural secretion." He proceeds, "There is no doubt that secretion from the adrenal medulla is subject to impulses delivered by the splanchnic nerves and there is no doubt that opening the abdominal cavity under anesthesia results in a discharge of impulses along these nerves. The adrenal glands, therefore, are continuously and abnormally stimulated if the abdomen is opened." If Bayliss and Starling had found that opening the abdomen caused the setting up of inhibitory impulses for the intestines, this would, of course, form a complication in the study of the intestinal movements under these conditions. Despite this, Cannon accepts their results as, so far as we are aware, everybody else does. But because he imagines that opening the abdomen sets up inhibitory impulses for the intestinal movements which, however, did not prevent Bayliss and Starling from making trustworthy observations, he concludes that our results on a totally different object, the adrenals, are completely vitiated by impulses set up in an altogether different group of nerve fibers, the epinephrin-secreting fibers, although neither he nor anybody else has ever shown that opening the abdomen influences them in the least. The only commentary we need make upon this is that Bayliss and Starling (7) do not call attention at all to the effect of opening the abdomen in causing the intestines to become absolutely motionless. On the contrary, they state that when they opened the abdomen in the warm-saline bath they found the intestines collapsed and absolutely motionless if the splanchnic nerves were intact. They drew the conclusion, not that

the opening of the abdomen caused the inhibitory impulses to the intestines to be set up but that these impulses were already descending the splanchnics before the abdomen was opened. Singularly enough, Cannon quotes their statement *verbatim:* "These facts suggest that in the *intact* animal, at any rate under the conditions of our experiment, tonic or reflex influences are continuously descending the splanchnic nerves, inhibiting the activity of the intestines." To be logical, then, he ought to conclude that since the impulses which pass to the intestines along the splanchnics are *not* called into existence by opening of the abdomen but are already present, impulses descending the splanchnics to the adrenals and sustaining a normal epinephrin output must also be present in the intact animal. If opening the abdomen so completely deranges the action of abdominal viscera, including the adrenals, a very large part of our supposed physiological knowledge must be wiped out. The truth is, of course, that we must neither assume in any particular case that opening of the abdomen is indifferent to the experiment nor that it absolutely contraindicates it, but must always test, as far as possible, whether and in what direction this operation, as well as the other experimental conditions, influence the result. We have pointed out elsewhere (22) that the relatively narrow range within which the output, (as measured by observers, including ourselves, who have employed methods correct in principle), varies with different anesthetics and different operations suggests strongly that what we term the normal or ordinary spontaneous output is not initiated or sustained by the trauma or the anesthesia.

To emphasize his criticism of our method of obtaining adrenal vein blood by opening the abdomen, Cannon states that the acceleration of the denervated heart, caused by stimulation of the central end of the sciatic, is hardly ever observed after opening the abdomen, although asphyxia still gives the reaction. He apparently considers this remark so important that he italicises the following sentence, "in the entire series of cases with opened abdomen there was only one in which sensory stimulation caused any effect ascribable to adrenal secretion." Since we have shown (8) that this heart reaction has no significance as an indication of augmented epinephrin output, the statement that acceleration on sciatic stimulation is rarely obtained after the abdomen has been opened would, even if true, have no bearing upon the question at issue. It is easy to demonstrate, however, that the reaction is readily obtained not only soon after the abdomen has been opened but long thereafter, and when the abdominal aorta, renal vessels and cava

have been tied or clipped off in making the cava pocket. Indeed, there was some indication that the reaction might be greater after clipping the abdominal aorta, presumably owing to the better blood flow in the nervous centers (see, e.g., protocol of cat 437 in the previous paper (8). There is plenty of evidence in the protocols of that paper that opening of the abdomen does not interfere with the reaction in question, and this is further illustrated here in figures 1 to 4.

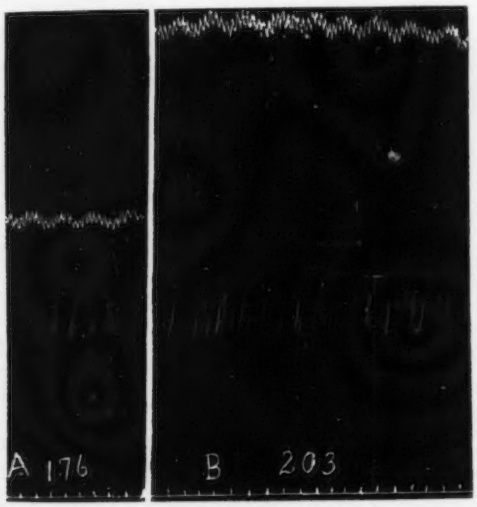

Fig. 1. Parts of blood pressure tracing from cat 175. *A*, before and *B*, a portion commencing 24 seconds after beginning sciatic stimulation; (abdomen opened, left splanchnic cut nearly 2 hours). In all figures line of zero pressure corresponds with time trace; time in seconds; numbers above time trace represent heart rate per minute.

The protocols of the experiments from which these tracings are taken have already been published (8). In figure 1 are reproduced two portions of the blood pressure tracing (from cat 175), one just before, *A*, and the other during stimulation of the sciatic, *B*. The pulse rate was increased by nearly 30 beats per minute. The abdomen had not only been opened almost 2 hours before, and the left splanchnic nerve cut, but the intestines had been purposely manipulated. The heart reaction had been obtained many times after opening the abdomen,

both by excitation of the sciatic and by excitation of the left splanchnic before this tracing was taken, but an excellent rise of pressure was still caused when either of these nerves was stimulated. Stimulation of the sciatic before the abdomen was opened and with both splanchnics intact gave an acceleration of 22 beats in one observation and 25 beats in another.

Figure 2 from another cat (177) shows an acceleration of almost 40 beats produced by sciatic stimulation after opening the abdomen and

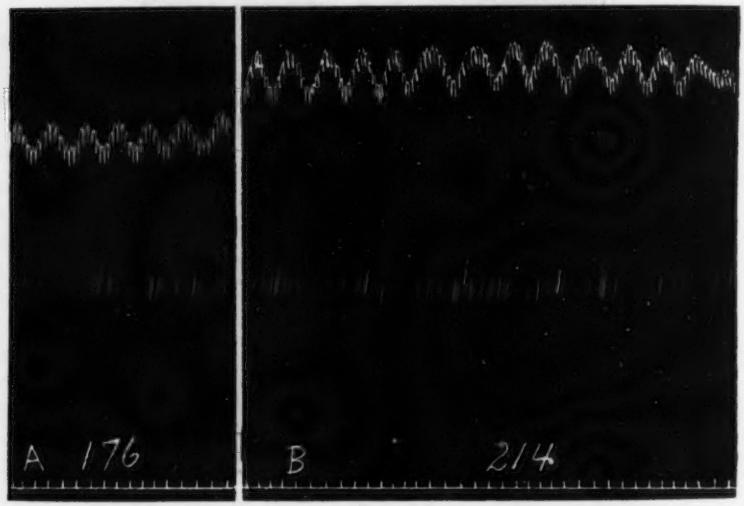

Fig. 2. Parts of blood pressure tracing from cat 177. *A*, before and *B*, a portion commencing 32 seconds after beginning of sciatic stimulation (abdomen open). Reduced one-nineteenth.

section of one splanchnic. Before the abdomen was opened, stimulation of the sciatic caused a maximum acceleration of 43 beats, with both splanchnics intact.

In figure 3 are reproduced portions of a curve (from cat 179) demonstrating a maximum acceleration of 34 beats, produced by sciatic stimulation before opening of the abdomen. One splanchnic had been previously cut extraperitoneally. After the abdomen was opened, a number of sciatic and splanchnic stimulations having been made in the meantime, stimulation of the sciatic caused an acceleration of 24

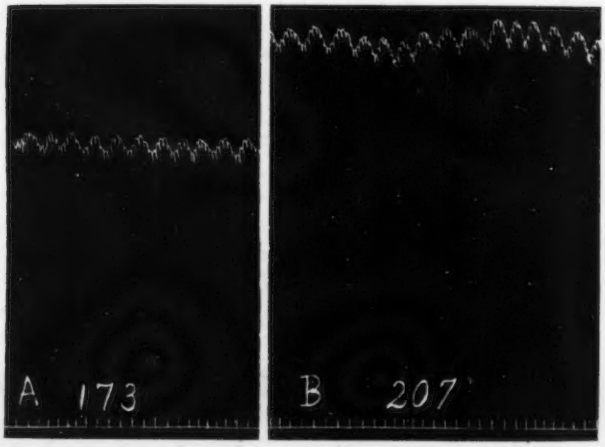

Fig. 3. Parts of blood pressure tracing from cat 179. *A*, before and *B*, a portion commencing 32 seconds after beginning of sciatic stimulation (left splanchnic cut).

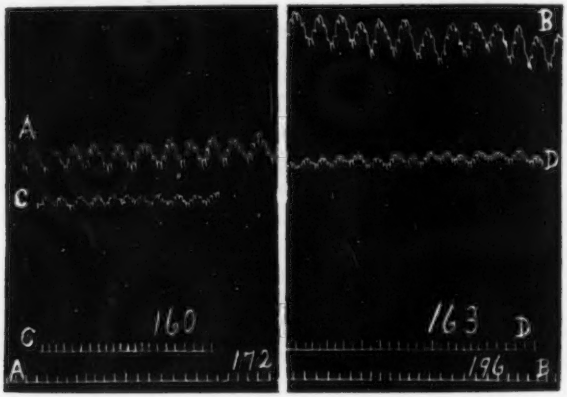

Fig. 4. Parts of blood pressure tracing from cat 179. *A*, before and *B*, a portion commencing 25 seconds after beginning of sciatic stimulation (left splanchnic cut and abdomen opened). *C*, before and *D*, a portion commencing 23 seconds after beginning of sciatic stimulation (both splanchnics cut).

beats (fig. 4, *A* and *B*). In this, as in all the other experiments, so long as sciatic stimulation gave a good rise of blood pressure, it gave a substantial acceleration of the heart. Later on in the experiment the second splanchnic was cut. The blood pressure fell to 44 mm. of mercury, and stimulation of the sciatic now caused little or no acceleration of the heart and only an insignificant rise of blood pressure (fig. 4, *C* and *D*).

If Cannon believes that the presence of the reaction indicates increased epinephrin output, it is incumbent upon him to explain why in adrenal blood collected at a time and under conditions when this reaction is well obtained, we are unable to detect, by a direct and sensitive method of assay (intestine segments), any sensible increase in the rate

Fig. 5. Parts of blood pressure tracing from cat 436. *A*, before asphyxia for 60 seconds; *B*, 7 seconds before end and 16 seconds after end of asphyxia; *C*, a portion commencing 40 seconds after end of asphyxia (abdominal aorta and renal vessels tied). Reduced to four-fifths.

of output. In the case of asphyxia it is still more necessary that he should explain why we cannot detect such great increases in the epinephrin output as he assumes to occur. For he admits that the acceleration of the denervated heart associated with asphyxia is not abolished by opening the abdomen. We can confirm this entirely, and can add that the reaction can also be obtained after ligation of the abdominal aorta, renal arteries and veins and other vessels, as done in the formation of a cava pocket for the collection of adrenal vein blood. This is illustrated in figure 5 (from cat 436).

Condensed protocol. Cat 436; male; weight 2.26 kgm.

Left superior cervical ganglion excised 28 days previously. Under urethane (6 grams) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation. The anesthesia was very deep and considerable depression of respiration and circulation was present from the start.

		Rate	Pressure
12:42 p.m.	Before sciatic stimulation	203	56
	6 seconds after beginning stimulation	206	62
	20 seconds after beginning stimulation	210	64
	40 seconds after beginning stimulation	204	64
12:52 p.m.	Before asphyxia for 45 seconds	135	26
	Just after beginning asphyxia	140	30
	10 seconds after beginning asphyxia	162	46
	26 seconds after beginning asphyxia	195	45
1:05 p.m.	Opened abdomen, tied abdominal aorta, renal arteries and veins		
1:10 p.m.	Before asphyxia for 45 seconds	184	49
	Just after beginning asphyxia	180	54
	15 seconds after beginning asphyxia	181	62
	Just after end of asphyxia	198	60
1:15 p.m.	Intravenous injection of 50 cc. Ringer's solution		
	Before Ringer injection	200	53
	After Ringer injection	204	80
1:35 p.m.	Before asphyxia for 60 seconds	194	66
	Just after beginning asphyxia	199	78
	30 seconds after beginning asphyxia	178	56
	Just after end of asphyxia	212	62
	20 seconds after end of asphyxia	219	53
	40 seconds after end of asphyxia	223	54
1:40 p.m.	Tied coeliac axis and superior mesenteric artery, clipped cava; hind leg and tail reflexes still present		
1:42 p.m.	Before asphyxia for 60 seconds	205	63
	During first 30 seconds of asphyxia	201	64
	Just after end of asphyxia	204	65
	15 seconds after end of asphyxia	218	60
	30 seconds after end of asphyxia	220	59
1:48 p.m.	Completed cava pocket		
1:49 p.m.	Before closure of pocket	208	56
	During closure of pocket	190	42
	Just after release of pocket blood	189	44
	15 seconds after release of pocket blood	215	58

In any case how does Cannon conceive asphyxia to act upon the epinephrin output? He says a short asphyxia acts through the nervous system, a longer period of asphyxia has a direct action. Now, why should the stimulating action of asphyxia on the part of the central nervous system which presides over the adrenal epinephrin secretion be abolished by tying the abdominal aorta? The effect of its ligation is to raise the blood pressure and therefore to keep up a more efficient circulation in the central nervous system and adrenals. How does this prevent the stimulation of the nerve centers by asphyxia? It does

not do so, and the reason we do not find an increased output of epinephrin with asphyxia must be that when tested by a quantitative method, the alleged increase is non-existent or too small to be detected.

The relation of the epinephrin output of the adrenals to the acceleration of the denervated heart associated with asphyxia. Cannon states that tying the lumbo-adrenal veins on both sides of the adrenals may not diminish in the least the acceleration of the heart produced by asphyxia of a given duration. This seems very good evidence that the reaction cannot be a quantitative reaction for epinephrin output. It is fully in accord with this conclusion that Cannon himself sometimes obtained a very large acceleration by asphyxia after section of both splanchnics. He endeavors to explain this by assuming that prolonged asphyxia stimulates the adrenal medullary cells directly. He brings forward no real evidence that this occurs. v. Anrep (9) states that after section of both splanchnics (in the dog) no reaction is elicited by asphyxia on the blood vessels of the denervated limb, and concludes that asphyxia has no direct action upon the adrenal medulla. Pearlman and Vincent (10), however, have not been able to obtain on the denervated limb any reaction in asphyxia which could be attributed to epinephrin. If asphyxia lasting for 90 seconds can stimulate the adrenal medulla directly so as to cause enough epinephrin to be given off to accelerate the heart by 68 beats a minute after section of the splanchnics, our failure to obtain evidence of this effect in adrenal vein blood directly assayed would be more puzzling than ever. For Cannon himself admits that the asphyxial acceleration with intact splanchnics is not interfered with by opening the abdomen, and how then should opening the abdomen abolish an action of asphyxia exerted directly upon the adrenal cells? Cannon's conclusion, that asphyxia stimulates directly the adrenal medulla, is simply a misinterpretation of his result that sometimes after division of both splanchnics asphyxia may cause a quickening of the denervated heart. Even if the heart reaction were specific for epinephrin, this would only indicate that some portion of the nerve supply of the adrenals might have been spared, and would not of itself prove a direct action. But as soon as it is shown that the reaction is not specific for epinephrin, and can be obtained after removal of the adrenals, the whole argument is seen to be baseless.

Cannon cites Czubalski (11) as having "adduced evidence that asphyxia if sufficiently prolonged may have a direct stimulating action on the adrenal medulla." The paper referred to is a preliminary note in which it is stated that even after section of the cord and bulb and also after division of the splanchnics

and vagi, a rise of blood pressure can be produced beginning usually at the end of the third or beginning of the fourth minute of asphyxia. The rise of blood pressure was very often accompanied by marked slowing of the heart. Czubalski attributes the increase of pressure to adrenalin liberated by the direct action of asphyxia on the adrenal medulla. The heart, of course, was isolated as regards its extrinsic innervation, and, according to Cannon, the characteristic response of the heart thus isolated to increased epinephrin output is an acceleration. It is curious that he should find confirmation of his result on the direct action of asphyxia on the adrenals in an effect upon the heart precisely the opposite of that which he associates with epinephrin. Czubalski's assertion that the general blood of a dog after asphyxia contains so much epinephrin that its presence is demonstrated by marked inhibition of a rabbit's intestine segment, when the defibrinated blood is made to displace "a nutritive solution," is of itself sufficient to show that his conclusions are not of any value. His statement that after removal of the adrenals asphyxia no longer causes a rise of blood pressure, is invalidated by the observations of Gley and Quinquaud (13).

In one of Cannon's experiments he obtained with an asphyxia lasting 90 seconds, which is what he considers a prolonged asphyxia, an acceleration of 28 beats before section of the splanchnics. After section of the splanchnics no acceleration was produced by an asphyxia of 60 seconds, but he does not state what acceleration, if any, was caused by an asphyxial period of the same duration as the control before splanchnotomy, so that there is no way of judging whether the section of the splanchnics in this case had anything to do with the negative result. If the asphyxial rise of pressure is a factor in the acceleration, it is easy to see that section of the splanchnics or injury to them in removal or ligation of the adrenals must interfere with the reaction, apart altogether from the interference with the epinephrin output. In the figure given by Cannon (fig. 2) there seems to have been no rise of pressure after removal of the adrenals till 45 seconds from the beginning of asphyxia, whereas before removal of the adrenals the curve shows a substantial rise of pressure before this, how much earlier it is impossible to say from the section of the curve reproduced.

It is unnecessary, however, to go into details of this kind. The best proof that Cannon's supposed demonstration of the augmenting effect of asphyxia upon the epinephrin output by means of the heart reaction is fallacious is that asphyxia can elicit marked acceleration in the absence of the adrenals.

Experiments showing that asphyxia can cause acceleration of the denervated heart in the absence of epinephrin from the adrenals. Several instances have been given in the previous paper (8). Thus in cat 438 the heart was beating at the rate of 175 a minute. The second adrenal had been removed 35 minutes previously by the abdominal route. Asphyxia was induced for 60 seconds and the heart rate rose to 225 beats a minute, the maximum acceleration being attained after resumption of respiration. The blood pressure was 64 mm. of mercury and

did not change much. Sciatic stimulation at this time caused little if any acceleration of the heart, although earlier in the experiment and after removal of the second adrenal it gave a good acceleration. In cat 440 after removal of the adrenals and exposure of the cervical cord, which was associated with a fall of blood pressure of 20 to 30 mm. of mercury, asphyxia for 45 seconds caused an acceleration of 13 beats during the asphyxial period and of 18 beats counting from the end of asphyxia, the blood pressure rising only slightly (from 84 to 88 mm. of mercury). Stimulation of the sciatic, which had caused good accelerations (as much as 42 beats a minute) after excision of the second adrenal, had at this time only a very slight effect upon the heart rate. With a subsequent asphyxia of 30 seconds, the heart rate diminished from 216 a minute before to 203 during the asphyxia, but recovered to the initial rate on stopping the asphyxia.

In a number of experiments the preliminary dissection for excluding the adrenals from the circulation was made by the abdominal route at the beginning of the experiment, and ligatures were placed but not tied. The abdomen was then closed. After one or more periods of asphyxia with the adrenals discharging, the ligatures were tied so as to occlude the adrenal arteries, the adrenal veins and the lumbar veins just before they cross the glands. The abdomen was closed and the effect of asphyxia on the heart rate was again observed. Finally the adrenals were excised by cutting between them and the ligatures. There was no bleeding and no further ligatures were required, showing that the glands had been completely excluded from the circulation. The protocol of cat 445 is given as an example.

Protocol. Cat 455; female; weight, 2.27 kgm. Under ether cut vago-sympathetics, excised stellate ganglia, opened abdomen and placed ligatures in position to occlude adrenal vessels but did not tie them.

		Rate	*Pressure*
12:52 p.m.	Before asphyxia (30 seconds)	284	130
	Just after end of asphyxia	313	118
	15 seconds after end of asphyxia	318	127
	30 seconds after end of asphyxia	304	126
12:55 p.m.	Before asphyxia (45 seconds)	268	125
	During first 15 seconds asphyxia	274	130
	During next 15 seconds asphyxia	278	114
	During next 15 seconds asphyxia	295	100
	Just after end of asphyxia	312	120
	20 seconds after end of asphyxia	323	132
	35 seconds after end of asphyxia	316	138

		Rate	*Pressure*
1:05 p.m.	Before asphyxia (60 seconds)	308	102
	During first 15 seconds asphyxia	303	105
	During next 15 seconds asphyxia	302	96
	Just after end of asphyxia	321	57
	15 seconds after end of asphyxia	335	72
	30 seconds after end of asphyxia	350	72
	50 seconds after end of asphyxia	334	76
1:12 p.m.	Tied off adrenal vessels		
1:14 p.m.	Before asphyxia (40 seconds)	283	102
	During first 12 seconds asphyxia	287	98
	Just after end of asphyxia	301	81
	15 seconds after end of asphyxia	321	95
	30 seconds after end of asphyxia	301	94
	45 seconds after end of asphyxia	298	94
1:18 p.m.	Before asphyxia (60 seconds)	283	95
	During last 15 seconds asphyxia	313	93
	Just after end of asphyxia	326	96
1:23 p.m.	Before asphyxia (60 seconds)	300	105
	During first 15 seconds asphyxia	300	109
	During next 15 seconds asphyxia	296	98
	During next 15 seconds asphyxia	308	98
	During next 15 seconds asphyxia	312	98
	Just after end of asphyxia	316	100
	15 seconds after end of asphyxia	310	100
	30 seconds after end of asphyxia	303	100
1:30 p.m.	Excised both adrenals; prepared central end of sciatic		
1:40 p.m.	Before asphyxia (35 seconds)	287	95
	During first 20 seconds asphyxia	285	98
	Just after end of asphyxia	280	64
	15 seconds after end of asphyxia	283	64
	30 seconds after end of asphyxia	284	64
	45 seconds after end of asphyxia	287	84
1:50 p.m.	Before sciatic stimulation (6 cm.)	264	67
	Just after end of stimulation	286	90
	15 seconds after end of stimulation	284	75
1:57 p.m.	Before asphyxia (60 seconds)	261	70
	During asphyxia	264	57
	Just after end of asphyxia	267	68
	20 seconds after end of asphxia	269	69
	35 seconds after end of asphyxia	270	72
2:06 p.m.	Before asphyxia (45 seconds)	238	52
	During first 15 seconds asphyxia	238	57
	During next 15 seconds asphyxia	238	50
	Just after end of asphyxia	249	48
	15 seconds after end of asphyxia	247	44
	30 seconds after end of asphyxia	248	44
	50 seconds after end of asphyxia	247	60

		Rate	*Pressure*
2:14 p.m.	Before sciatic stimulation	238	49
	During first 15 seconds stimulation	239	63
	During next 10 seconds stimulation	242	64
	During next 10 seconds stimulation	246	65
	Just after end of stimulation	242	64
2:32 p.m.	Before asphyxia (45 seconds)	244	62
	During first 25 seconds asphyxia	245	54
	Just after end of asphyxia	242	45
	20 seconds after end of asphyxia	240	49

It will be seen that ligation of the adrenals did not interfere with the acceleration of the denervated heart induced by asphyxia. The first asphyxia after ligation of the glands gave an acceleration of 38 beats, the second an acceleration of 43 beats per minute. The third asphyxia gave an acceleration of 16 beats, but the initial rate was already 300, which was 17 beats greater than before either of the two previous asphyxial periods. After actual removal of the adrenals in this experiment the maximum acceleration caused by asphyxia was slight (only 9 beats per minute). This, however, can have nothing to do with loss of epinephrin output since the glands had been entirely excluded from the circulation by the ligation.

We have had abundant evidence, if evidence were needed, that repeated asphyxiation is not an indifferent procedure for the heart. A heart which at first has responded by an acceleration during the asphyxial period, and usually a still greater acceleration immediately thereafter, may later on respond by some diminution in the pulse rate, becoming more manifest toward the end of the asphyxia, and succeeded by an increase on stopping the asphyxia, which may carry the rate beyond the initial value or not. In figure 2 of Cannon's paper (4) the heart rate toward the end of asphyxia (after the adrenals were tied off) was slower by 6 or 7 beats a minute than before the asphyxia. It then increased to the initial rate after respiration was resumed. As only a small portion of the curve is reproduced, it does not show whether later on the rate went beyond the initial value or not. When successive periods of asphyxia are superimposed upon a steadily declining blood pressure in the course of an experiment the loss or diminution of the heart reaction is apt to be particularly evident.

A good acceleration was still given when the sciatic was stimulated after excision of the adrenals, accompanied by a fair rise of pressure. It is scarcely necessary to point out that it is impossible to reconcile these results with Cannon's conclusion that the acceleration of the denervated heart is an index of the increased output of epinephrin from the adrenals caused by stimulation of the sciatic and by asphyxia. In cat 455 the heart rate after section of the vago-sympathetics and excision of the stellate ganglia was exceptionally great.

In the next experiment to be cited the heart rate was unusually low, not much more than half the rate in cat 455 and the blood pressure was also only about half.

Protocol. Cat 453; male; weight, 2.51 kgm. Under urethane (5 grams) cut vago-sympathetics, excised stellate ganglia, opened abdomen and placed ligatures in position to occlude adrenal vessels but did not tie them.

		Rate	*Pressure*
11:35 a.m.	Before asphyxia (45 seconds)	150	66
	During first 20 seconds asphyxia	152	66
	During next 20 seconds asphyxia	152	72
	Just after end of asphyxia	153	76
	20 seconds after end of asphyxia	155	68
	35 seconds after end of asphyxia	156	71
11:38 a.m.	Before asphyxia (60 seconds)	153	63
	During first 15 seconds asphyxia	151	63
	During next 20 seconds asphyxia	151	69
	During next 15 seconds asphyxia	157	85
	Just after end of asphyxia	166	77
	25 seconds after end of asphyxia	166	80
11:45 a.m.	Tied off adrenal vessels		
11:46 a.m.	Before asphyxia (60 seconds)	143	57
	During first 20 seconds asphyxia	144	60
	During next 30 seconds asphyxia	147	82
	Just after end of asphyxia	151	66
	25 seconds after end of asphyxia	153	70
11:50 a.m.	Before asphyxia (85 seconds)	143	59
	During first 30 seconds asphyxia	145	68
	During next 30 seconds asphyxia	146	76
	During next 20 seconds asphyxia	152	64
	Just after end of asphyxia	159	76
	25 seconds after end of asphyxia	160	74
	40 seconds after end of asphyxia	156	78
12:00 m.	Prepared central end of sciatic for stimulation		
12:02 p.m.	Before sciatic stimulation (5 cm.)	153	63
	During sciatic stimulation	174	106
	Just after end of stimulation	181	75
	25 seconds after end of stimulation	171	75
12:10 p.m.	Before asphyxia (55 seconds)	157	62
	During first 25 seconds asphyxia	158	64
	During next 30 seconds asphyxia	163	70
	Just after end of asphyxia	163	56
	20 seconds after end of asphyxia	170	63
12:15 p.m.	Excised both adrenals		
12:16 p.m.	Before sciatic stimulation (5 cm.)	156	57
	During first 20 seconds stimulation	166	77
	During next 20 seconds stimulation	183	72
	Just after end of stimulation	171	53

		Rate	*Pressure*
12:22 p.m.	Before asphyxia (60 seconds)	150	51
	During first 20 seconds asphyxia	150	54
	During next 20 seconds asphyxia	151	60
	During next 15 seconds asphyxia	157	42
	Just after end of asphyxia	153	41
	30 seconds after end of asphyxia	155	47
12:30 p.m.	Two more observations with asphyxia gave in the first a maximum acceleration of 6 beats, in the other none; sciatic stimulation caused no acceleration; blood pressure had fallen to 48 mm., and during asphyxia to 33 mm. of mercury.		

The greatest acceleration caused by asphyxia in cat 453 before ligation of the adrenals was 13 beats a minute; after ligation it was 17 beats a minute. After excision of the glands, already completely excluded from the circulation, the greatest acceleration obtained with asphyxia was 7 beats per minute, and at the end of the experiment practically no acceleration was caused. It is obvious that the absence of the reaction in the last observation could have nothing whatever to do with the absence of the epinephrin output of the adrenals. Sciatic stimulation before excision of the adrenals, but after their exclusion from the circulation, gave an acceleration of 28 beats, and after excision of the glands an acceleration of 27 beats per minute. How is it possible to maintain that these accelerations are due to increased output of epinephrin from the adrenals, or that the failure to obtain them is due to elimination of the epinephrin output? The experiment proves clearly that the acceleration caused by asphyxia cannot be due to the same factors as the acceleration caused by sciatic stimulation, since the latter is unchanged after excision of the adrenals. In other experiments an asphyxial acceleration has been obtained at a time when little or no acceleration was elicited by stimulation of the sciatic. Figure 6 gives portions of the blood pressure curve from cat 453, showing the effect of stimulating the sciatic before and after excision of the adrenals and the effect of asphyxia after the excision.

In figures 7 and 8 are reproduced portions of the tracings from cat 456, to show the effect of asphyxia before the adrenals were tied off (maximum acceleration 22 beats per minute), after the adrenals were tied off (maximum acceleration 25 beats per minute), after the adrenals were excised (maximum acceleration 13 beats a minute), and later on (no acceleration). As shown in the protocol, ligation of the adrenals did not alter the position of the maximum acceleration any more than its absolute amount. Counts of successive portions of the curves

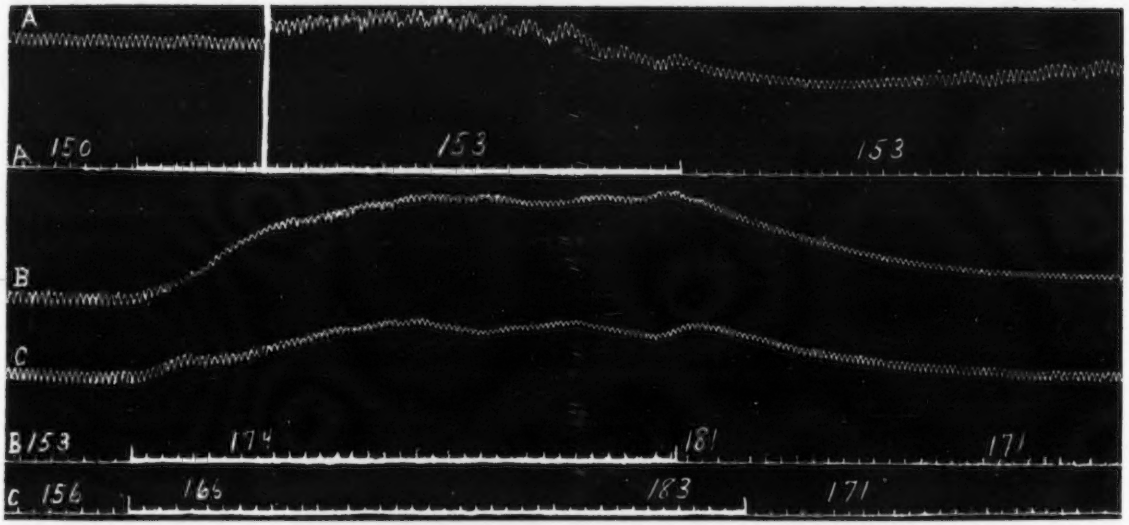

Fig. 6. Parts of blood pressure tracing from cat 453. *A*, asphyxia for 60 seconds, after excision of adrenals (26 seconds cut out of trace to save space); *B*, sciatic stimulation before, and *C*, after excision of adrenals. Reduced to three-fourths.

are given to establish this point, which is no more in harmony with the view that the acceleration is an index of epinephrin output than is the possibility of eliciting an undiminished acceleration after exclusion of the adrenals. Naturally, when, after ligation of the vessels, asphyxia was associated with a considerable fall of blood pressure no acceleration might be seen until respiration was resumed (Fig. 7). After re-

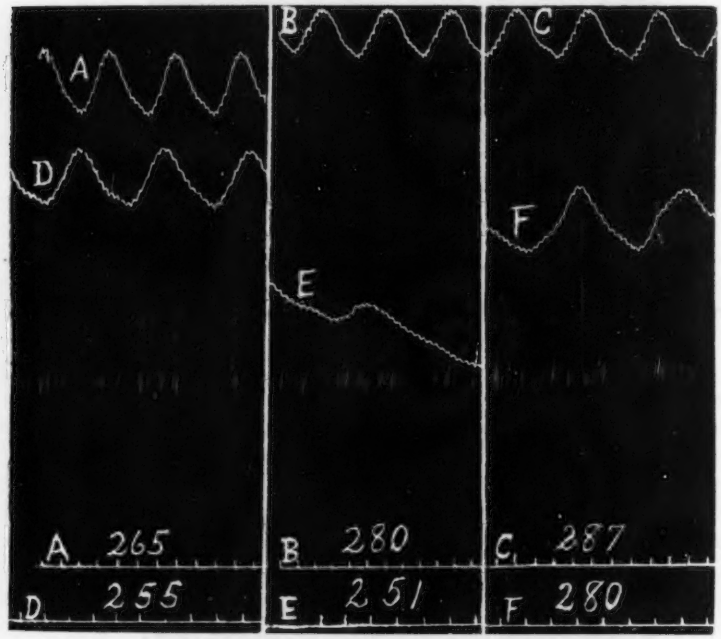

Fig. 7. Parts of blood pressure tracing from cat 456. *A*, before asphyxia, *B*, a portion just before end, and *C*, a portion commencing 18 seconds after end of asphyxia for 45 seconds, before tying adrenal vessels. *D*, before asphyxia, *E*, a portion just before end of asphyxia and *F*, a portion commencing 27 seconds after end of asphyxia for 45 seconds, after tying adrenal vessels.

peated asphyxiation the effect on the heart rate disappeared, although sciatic stimulation still caused a moderate acceleration (10 beats per minute). Again, it would be absurd to attribute the failure of the asphyxia reaction to elimination of the adrenals, since they were already

eliminated by the ligation and were cut out without bleeding, and since a fair acceleration was caused by asphyxia for some time after their removal.

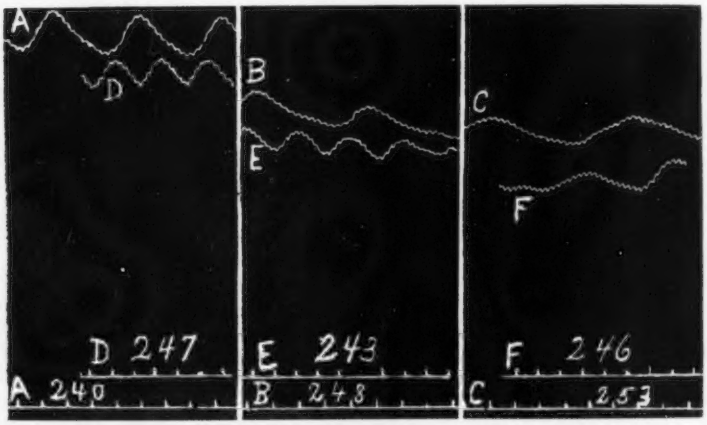

Fig. 8. Parts of blood pressure tracing from cat 456. *A*, before asphyxia, *B*, a portion just before end and *C*, a portion commencing 20 seconds after end of asphyxia for 45 seconds, after excision of adrenals. *D*, before asphyxia, *E*, a portion just before end and *F*, a portion commencing 21 seconds after end of asphyxia for 60 seconds, nearly half an hour after excision of adrenals.

Protocol. Cat 456; young female; weight, 1.55 kgm. Under urethane (3 grams) sectioned vago-sympathetics, excised stellate ganglia, opened abdomen and placed ligatures in position to occlude adrenal vessels but did not tie them.

		Rate	*Pressure*
11:53 a.m.	Before asphyxia (45 seconds)	265	145
	During first 15 seconds asphyxia	270	156
	During next 30 seconds asphyxia	278	163
	Just after end of asphyxia	282	161
	15 seconds after end of asphyxia	286	159
	30 seconds after end of asphyxia	287	156
	50 seconds after end of asphyxia	283	150
11:58 a.m.	Tied off adrenal vessels		
12:00 m.	Before asphyxia (45 seconds)	255	130
	During first 15 seconds asphyxia	260	132
	During next 15 seconds asphyxia	259	114
	During next 15 seconds asphyxia	252	80
	Just after end of asphyxia	269	108
	15 seconds after end of asphyxia	278	114
	30 seconds after end of asphyxia	279	126
	45 seconds after end of asphyxia	280	126

		Rate	Pressure
12:10 p.m.	Excised both adrenals		
12:12 p.m.	Before asphyxia (45 seconds)	244	111
	During first 15 seconds asphyxia	245	116
	During next 20 seconds asphyxia	245	102
	During next 10 seconds asphyxia	247	94
	Just after end of asphyxia	251	94
	20 seconds after end of asphyxia	253	98
	40 seconds after end of asphyxia	253	98
12:15 p.m.	Before asphyxia (45 seconds)	240	110
	During first 20 seconds asphyxia	244	100
	During next 20 seconds asphyxia	249	88
	Just after end of asphyxia	248	80
	20 seconds after end of asphyxia	253	88
	40 seconds after end of asphyxia	252	92
12:18 p.m.	Prepared central end of sciatic for stimulation		
12:20 p.m.	Before sciatic stimulation (6 cm.)	240	88
	During sciatic stimulation	252	140
	Just after end of stimulation	251	108
12:23 p.m.	Before asphyxia (through 3 ft. tube) (60 seconds)	239	110
	During first 20 seconds asphyxia	238	103
	During next 20 seconds asphyxia	239	90
	During next 20 seconds asphyxia	242	80
	Just after end of asphyxia	241	67
	20 seconds after end of asphyxia	244	83
	45 seconds after end of asphyxia	247	92
12:30 p.m.	Before asphyxia (105 seconds) interrupted by 5 seconds respiration each half minute	240	96
	During first 20 seconds asphyxia	242	90
	During next 20 seconds asphyxia	243	85
	During next 20 seconds asphyxia	245	86
	During next 15 seconds asphyxia	244	80
	During next 20 seconds asphyxia	248	82
	Just after end of asphyxia	246	80
12:34 p.m.	Before sciatic stimulation (5 cm.)	242	88
	During first 15 seconds stimulation	247	106
	During next 20 seconds stimulation	252	120
	Just after end of stimulation	250	102
	20 seconds after end of stimulation	247	90
12:37 p.m.	Before asphyxia (60 seconds)	247	89
	During first 20 seconds asphyxia	245	76
	Just after end of asphyxia	243	57
	20 seconds after end of asphyxia	243	68
	45 seconds after end of asphyxia	246	72
12:42 p.m.	Before sciatic stimulation (6 cm.)	242	78
	During first 15 seconds stimulation	245	100
	During next 20 seconds stimulation	252	117
	Just after end of stimulation	252	99
	20 seconds after end of stimulation	250	86

		Rate	Pressure
12:50 p.m.	Before asphyxia (80 seconds)	250	72
	During first 20 seconds asphyxia	251	82
	During next 20 seconds asphyxia	251	66
	During next 20 seconds asphyxia	252	64
	Just after end of asphyxia	248	58

In one cat (452) we obtained practically no acceleration with asphyxia (maximum increase in heart rate 5 beats per minute) after removal of the adrenals, although before removal a maximum acceleration of 42 beats had been got in one observation. In the very first observation, however, with a somewhat shorter asphyxial period the acceleration was very slight (6 beats per minute). The blood pressure was exceedingly high after denervation of the heart (214 mm. of mercury). After removal of the adrenals by the abdominal route it sank to 114 mm. of mercury, and the pulse rate declined nearly 40 beats a minute.

Condensed protocol. Cat 452; old male; weight, 4.17 kgm. Under urethane (5.5 grams) cut vago-sympathetics and excised stellate ganglia

		Rate	Pressure
11:30 a.m.	Before asphyxia (30 seconds)	266	214
	During first 15 seconds asphyxia	260	214
	During next 15 seconds asphyxia	253	214
	Just after end of asphyxia	270	207
	15 seconds after end of asphyxia	272	215
	35 seconds after end of asphyxia	265	206
11:35 a.m.	Before asphyxia (45 seconds)	258	190
	During first 20 seconds asphyxia	258	207
	During next 15 seconds asphyxia	256	220
	During next 10 seconds asphyxia	271	205
	Just after end of asphyxia	295	196
	20 seconds after end of asphyxia	300	203
	40 seconds after end of asphyxia	284	200
12:07 p.m.	Excised both adrenals (extraperitoneally)		
12:08 p.m.	Before asphyxia (45 seconds)	222	114
	During first 20 seconds asphyxia	222	118
	During next 25 seconds asphyxia	219	121
	Just after end of asphyxia	222	102
	20 seconds after end of asphyxia	224	115
	40 seconds after end of asphyxia	223	115
12:14 p.m.	Before asphyxia (30 seconds)	218	110
	During first 15 seconds asphyxia	223	118
	During next 15 seconds asphyxia	223	127
	Just after end of asphyxia	220	110
	20 seconds after end of asphyxia	220	120
	35 seconds after end of asphyxia	223	120

12:18 to 12:35 p.m. Three more observations with asphyxia (60 to 90 seconds) gave a small drop in the rate during the asphyxia; the blood pressure had fallen to 84 at the end of the experiment.

We do not think anybody who has studied our previous results will attribute the failure of the reaction in this experiment, after adrenalectomy, to elimination of the epinephrin output of the adrenals. But if by any chance a reader chooses to conclude, as apparently Doctor Cannon would do, that the drop of 36 beats a minute in the rate after removal of the glands is due to the absence of the epinephrin previously being given off from the adrenals (average 0.0002 mgm. per kgm. per minute in etherized cats) he will have difficulty in seeing why an increase in the output to many times this amount, with asphyxia, should be necessary to cause an acceleration of 42 beats a minute before the adrenalectomy, and why it should be impossible that a redistribution of the epinephrin without any increase in the rate of output might be responsible for the acceleration, even if asphyxia produces no change in the susceptibility of the heart to the action of epinephrin. The only possible conclusion, however, from our experiments is that whether the epinephrin takes any share in the acceleration associated with asphyxia or not, the reaction cannot be due solely to the adrenal epinephrin, and there is no evidence that epinephrin is concerned in it at all.

As has been pointed out in the previous paper (8), our proof that the increase in the heart rate caused by stimulation of the sciatic cannot be an index of an increased epinephrin output, does not entail the obligation to explain the acceleration, which is probably a complicated reaction. Still less do we feel bound to explain the asphyxial acceleration, which is possibly more complicated still. It is for Doctor Cannon, who brings forward the reaction as a quantitative test for changes in the epinephrin output, to exclude the other possible factors. We have suggested, however, (12) one possibility which must be controlled in so far as epinephrin may be a factor at all, namely, that asphyxia might render the heart more sensitive to such amounts of epinephrin as were being given off before the asphyxia was induced. Since we have shown that asphyxia in the absence of the adrenals can cause acceleration, it seems obvious that the accelerating action of a given dose of epinephrin might be reinforced by asphyxia. In that paper (12) we stated that it is not permissible to make quantitative comparisons on the effect of such a condition as asphyxia, using a test object like the heart well supplied with oxygen in one observation but asphyxiated in the comparison observation, without controlling any possible effect produced by the asphyxia upon the reactivity of the test object itself. There was every reason to point this out, as this factor is habitually

neglected by certain writers, who have worked with test objects *in situ*, although it must be taken account of before their results can be used at all.

Cannon now endeavors to control the possibility that asphyxia might alter the reactivity of the heart to one and the same dose of epinephrin, so that a reaction might be obtained simulating an increased output when the output was really unchanged. Although the question loses much of its interest, as a question in the technique of estimating epinephrin output, when it is known that the heart reaction relied upon by Cannon to demonstrate increased output is readily obtained in the absence of the adrenals, it may nevertheless be noted how Cannon takes account of the factor in question. He reproduces a curve taken while adrenalin, to the amount of 0.08 mgm. per minute, was being continuously injected into the veins of a cat. This is, for an average cat under ether, 200 times the mean rate of output of epinephrin under the conditions of our experiments, which according to Cannon may artificially increase the output to an "unsurpassable limit." The concentration of epinephrin in the blood passing through the coronary circulation must, therefore, have been much greater than could probably ever be sustained by the output from the adrenals, even if, according to Cannon, this were greatly increased by excitation of sensory nerves or asphyxia. The increase in the heart rate, however, was only 40 beats (from 132 to 172 per minute), an acceleration of the same order of magnitude as may be given by sciatic stimulation or asphyxia alone, without any artificial injection of adrenalin. Now, since the amount of adrenalin injected is, according to Cannon's own data, far greater than that liberated from the adrenals by asphyxia, it must be assumed that the acceleration was maximal before asphyxia was allowed to act. How can a possible change in the reactivity of the heart to epinephrin under the influence of asphyxia be demonstrated if the heart is already making its maximum effort in response to an enormous dose of injected adrenalin? The slowing of the heart, with the drop of pressure as the asphyxia continued, does not indicate that asphyxia of moderate duration renders the heart less sensitive to epinephrin, but is a very common phenomenon in asphyxia when no adrenalin has been injected, and when none can be coming from the adrenals. Epinephrin liberated at the ordinary rate may possibly enable the heart to resist better, and for a longer time, the depressing influence of asphyxia, and to respond better to the factors responsible for the acceleration either during the asphyxial period, or when respiration is resumed.

Another factor in the asphyxial acceleration, if epinephrin takes any share in it, may be, as already pointed out in the case of the acceleration caused by stimulation of sensory nerves (8), the redistribution of the blood associated with the vascular changes, which may result in a greater quantity of epinephrin per unit of time or a greater concentration of it being supplied to the coronary circulation, without any increase having occurred in the rate of output from the adrenals.

In another paper (12) we cited some evidence that certain reactions may be produced by epinephrin in this way, for instance, an experiment of v. Anrep (9) in which he shows that "if one splanchnic nerve is intact while the suprarenal on the other side is extirpated, stimulation of the splanchnic nerve on the side of the extirpated suprarenal may still cause constriction of a denervated limb. Only after the other splanchnic nerve is cut does the constriction disappear and the limb react passively to the change of blood pressure." His interpretation of this result is that "this is due to the fact that stimulation even of the peripheral end of the splanchnic excites a certain number of afferent nerves, so that there may be a reflex excitation of the suprarenal of the other side through the intact splanchnic nerve." There is no evidence that stimulation of the peripheral end of the splanchnic nerve can affect reflexly the rate of epinephrin secretion from the other adrenal and excellent evidence against it. The true explanation, we believe, is that so far as epinephrin is a factor in v. Anrep's reaction, the constriction seen under the circumstances described is due to the shunting through the denervated limb of more of the epinephrin being given off at the ordinary rate from the other adrenal. Cannon has suggested that in the cat (efferent) adrenin secretory fibers may occasionally pass from one splanchnic to the opposite adrenal, but he produces no proof of such a crossing. We have never seen evidence of any increase in the output of one adrenal when the opposite splanchnic was stimulated, or any evidence of diminished output when the opposite splanchnic was cut. Elliott's results on the protection of the epinephrin store from depletion in the adrenal whose nerves are cut, as compared with its fellow, could hardly have been what they were if both glands were innervated from each splanchnic. In the dog, v. Anrep could scarcely have failed to obtain the limb reaction in the experiment mentioned, after section of the opposite splanchnic, if there was a crossing of efferent fibers from the stimulated splanchnic to the other gland.

Redistribution of the blood owing to vasomotor changes is certainly not the only factor, if it is a factor, in the asphyxial acceleration, and in most of our experiments the rise of pressure produced by asphyxia after denervation of the heart was not great. Good accelerations were obtained with little or no increase of pressure, and the maximum acceleration was generally found when the pressure had fallen again, or, although rising after resumption of respiration, was still below the initial value. There was no obvious difference in this regard whether the adrenals had been excluded or not. Accordingly, such an experiment as Cannon illustrates in figure 7 of his paper (4) has no bearing on the question whether acceleration of the heart caused by asphyxia is due to increased epinephrin output. He denervated the heart, tied the limb and carotid arteries and severed the mesenteric nerves, and still got an increase in heart rate from 180 to 212 beats per minute after asphyxia, "with no previous noteworthy change in blood pressure." The pressure rose about 25 mm. of mercury after stopping the asphyxia,

and it was at this time that the maximum acceleration was counted. But how can this be a proof that asphyxia augments the output of epinephrin since equally large accelerations have been obtained by us in the absence of any epinephrin discharge?

As regards Cannon's method of demonstrating adrenal secretion by the rise of pressure caused by asphyxia after tying the carotid and limb arteries and "denervating the splanchnic area," it may be noted that the curve reproduced in figure 3 of his paper shows a slight elevation (less than 10 mm. of mercury) after the first minute of asphyxia, and a very large rise (at least 110 mm. of mercury) after resumption of respiration at the end of two minutes of asphyxia. He attributes both rises to the action of an increased output of epinephrin, but unfortunately does not state how much epinephrin would have been required to raise the pressure by 110 mm. of mercury. If such an enormous outpouring of epinephrin as would seem necessary to cause an effect of this kind were actually induced by asphyxia, it is impossible to see why we always missed it in collecting adrenal vein blood. Our operation was assuredly no more "severe" than Cannon's, the abdomen was opened by him also, and he states that the method yielded constant results so far as the belated influence of asphyxia was concerned, although "it was commonly disappointing as a means of demonstrating the early influence of asphyxia." If asphyxia can cause the liberation of so much epinephrin that a rise of pressure of this order of magnitude can be produced, it is also unintelligible that the careful experiments of Gley and Quinquaud (13) should not have revealed a striking difference in the effects of asphyxia on the blood pressure curve before and after eliminating the epinephrin output. They found that the curves were practically identical. Cannon's statement that after tying off the adrenals the rises of pressure did not occur, may mean nothing more than that the considerably lower blood pressure (during asphyxia it fell to little over 25 mm. of mercury) had so injured the heart that it could not respond to the resumption of respiration by such an increased action as, in the restricted circulation, would cause a marked rise of blood pressure, whereas, with the better circulation before exclusion of the adrenals it could do so.

These suggestions as to possible ways in which epinephrin may be a factor in the asphyxial acceleration of the denervated heart, without any increase in its rate of output having occurred, are not intended to imply that there is evidence that epinephrin takes any share in the acceleration. Our experiments show conclusively that it cannot be

the sole factor, if it is a factor at all, since marked accelerations can be obtained after exclusion or removal of the adrenals. As to the mechanism of the acceleration, we do not propose to enter further into a discussion of this reaction, in which direct effects of asphyxia upon the heart and indirect effects produced through the changed quality and quantity of the blood supplied to it may be intermingled. Our purpose was accomplished as soon as the statement that the reaction can be used to demonstrate an increased output of epinephrin during asphyxia was shown to be without foundation. Still less than in the case of the acceleration caused by sciatic stimulation can we imagine how such a reaction could be employed to measure quantitatively the rate at which epinephrin is given off by the adrenals.

Knowlton and Starling, working with the heart-lung preparation, have shown that the rate of the isolated mammalian heart is very sensitive to changes of temperature, although not altered by marked variations of arterial or venous pressure. But we have not included change of temperature of the blood coming to the heart during asphyxia or sensory stimulation, or change of temperature of the heart directly produced in asphyxia by stoppage of the heat loss through the respiratory tract, among the factors possibly concerned in the reaction because there is no evidence that under our experimental conditions this could have had any appreciable influence.

Cannon states that "the completely denervated heart can be used as an indicator of adrenal secretion in testing the influence of emotional excitement quite as well as in testing the influence of sensory stimulation and asphyxia." We have shown that it cannot be used at all in the case of sensory stimulation or asphyxia, and as, according to Cannon, it has the same value as a test for the influence of emotional excitement, we do not judge it necessary to discuss the matter here at any length.

Experiments on emotional excitement are necessarily less satisfactory than on sciatic stimulation or asphyxia because the animal must not be anesthetized. As a matter of fact, the evidence adduced by Cannon is extremely scanty and, in our opinion, will not bear examination. In one case, for instance, after removal of the left adrenal gland and section of the right splanchnic nerve in the thorax on the previous day an increase of 42 beats a minute was observed under excitement. In another case after a similar operation an increase of approximately 28 beats a minute was seen during excitement. These accelerations are of the same order of magnitude as those seen with both adrenals and their

nervous supply intact. How is it possible to consider a reaction as a quantitative test of the rate of epinephrin output which is given as well with one adrenal removed and the innervation of the other crippled as when both are normally discharging epinephrin? These animals had had many hours to recover from the effects of the anesthetic and operation and, therefore, gave a good heart reaction with excitement, a reaction which probably had little if anything to do with the assuredly diminished output of epinephrin from the remaining adrenal. That after removal of the remaining adrenal the reaction was not obtained when the animal was allowed to recover from etherization, is no proof that the failure of the reaction was due to the suppression of the residual epinephrin output of the gland, which was already largely denervated. The negative result is far more likely to have been due to the general condition of the animal just recovering from a second etherization and a second operation.

The only other experiment mentioned by Cannon is one in which excitement increased the heart rate from 217 to 255 beats per minute with the adrenals intact. Two days after this observation the adrenals were removed, of course under anesthesia, and about 5 hours thereafter excitement caused the rate to increase from 217 to 221 beats a minute. The conclusion is drawn that the difference in the result before and after the adrenalectomy is due to the loss of the epinephrin secretion. The possibility that the general condition of the animal after the adrenalectomy might be less favorable to eliciting a large acceleration is not considered. It must again be pointed out, as in the case of sciatic stimulation and asphyxia, that even if it had been proved that the failure of the reaction after removal of the adrenals was due to the absence of the adrenal epinephrin output and to this alone, the conclusion would not be justified that emotional excitement increases the rate at which epinephrin is liberated into the blood, until it was shown that the changes in the amount and concentration of the epinephrin passing through the coronary system, occasioned by the vascular effects of the excitement, were insufficient to account for the observed accelerations. We should have considered it a mere accident that the heart rate was the same (217 beats a minute) after removal of the adrenals as two days before. Cannon, however, believes that this "is an indication, and the first reliable one, that under quiet, peaceful conditions there is no adrenal secretion or a secretion so slight as not to affect the denervated heart, an extremely sensitive indicator." He argues that if there was a secretion of the magnitude observed by us there ought to have been a drop in the pulse rate when this secretion was eliminated. Doctor Cannon has not mentioned what the pulse rate was just before the adrenalectomy, so we do not know whether there was a decline in the rate or not after removal of the glands. We have, however, presented evidence both in the preceding paper (8) and in this, that other factors than the loss of epinephrin are concerned in the diminution of the pulse rate which usually follows removal of the adrenals and that sometimes that operation, or an equivalent interference with the epinephrin

output may not be followed by any diminution in the rate, while after exclusion of the adrenals a marked diminution may be caused by operative and other procedures associated with a fall of blood pressure.

The catheter method. Cannon sums up his work with the heart reaction by the declaration that "the results obtained with the isolated heart used as an indicator of adrenal secretion confirm in every respect the results obtained eight years ago by the catheter method." We have shown what kind of confirmation the isolated heart used as an indicator of adrenal secretion can lend to any other method, it could only render it suspect. Since, however, despite their confirmation by the isolated heart reaction, the results obtained by Cannon with the catheter method might be correct, we shall take this occasion to examine them on their merits in somewhat greater detail than has hitherto been done. This series of papers by Cannon and his co-workers deals with the influence of emotional excitement, asphyxia, stimulation of sensory nerves and some drugs upon the rate of epinephrin output. He collected blood from the inferior vena cava above the level of the adrenal veins by means of a catheter pushed up through the femoral vein. According to him, blood obtained from this region in cats in the absence of excitement, asphyxia, etc., does not cause inhibition of cat's intestine strips or rabbit's intestine segments, whereas blood obtained during or after the action of these factors causes marked inhibition, due to the outpouring of epinephrim. After emotional excitement lasting for 10 minutes or more, so much epinephrin has been or is being liberated that it can be detected in the general venous blood (femoral vein), after passing not only through the lungs but through the systemic capillaries. It may be remarked that if this is correct, it ought not infrequently to be possible to detect epinephrin in blood collected from patients or animals by puncture of a vein. For much excitement may attend even so small an operation. But no satisfactory evidence has ever been brought forward that detectable concentrations of epinephrin exist in the general venous blood. To account for the extraordinary output of epinephrin which would be necessary in order that it should be clearly detected in femoral vein blood, Cannon suggests that adrenalin liberated into the blood stimulates the adrenals to further increase the output. "Thus the more marked effect as time passes (see fig. 3) may be due not only to further excitement, but in part to an autogenous continuance of adrenal secretion. Thus also the persistence of the emotional state after the exciting object has disappeared can be explained. Indeed it was the lasting effect of excitement on digestive processes

which suggested this investigation." In support of this idea he appeals to a statement of Elliott's (14) that adrenalin itself causes depletion of the epinephrin store of the adrenals. Elliott himself later withdrew this statement (15), which in any case would not prove that adrenalin increased the output of epinephrin.

Cannon complains that "authors have written as if Cannon had been attempting to support the idea that emotional experiences were dependent upon circulating adrenin. Thus Stewart and Rogoff report, as if the matter had been questioned, that all signs of fright can be elicited by administering morphine to a cat with one adrenal removed and the other denervated." This subject is not referred to at all in the paper quoted by Cannon. In another paper (26), we are discussing the depletion of the epinephrin store produced by morphine, which Elliott (15) suggests is directly due to the fright caused by the drug. In this connection we say "That signs which might be interpreted as those of fright are present in cats under morphine is, of course, not doubtful. Whether this interpretation is correct might be difficult to decide, and does not concern us here. It is, however, of interest to note that epinephrin seems to have nothing to do with those signs. The signs of morphine fright can all be elicited by administering morphine to a cat in which one adrenal has been removed and the splanchnic supply of the other cut and in which accordingly no liberation of epinephrin through the splanchnics takes place." How can this be spoken of as "writing as if *Cannon* had been attempting," etc.? As a matter of fact, in that paper we were not discussing Cannon's psychological theories at all. We do not see that there was any impropriety in publishing observations showing that signs, which might be interpreted as those of fright, can be elicited by morphine in cats whose epinephrin output has been abolished or markedly diminished, even if we had been aware, which of course we could not be, that this detail in the pharmacology of the drug would seem self-evident to Cannon.

Cannon complains further that "Rogoff points out that the secretion of sufficient adrenin to produce symptoms of fright would be impossible, again as if any claim had been made that these symptoms were due to secreted adrenin." What Rogoff did say was: "In some of the cats (after removal of one adrenal and denervation of the other) it was found that the liberation of epinephrin from the adrenals was so interfered with by this operation that there could not have been one-thousandth of the normal liberation, if any epinephrin was being given off from the glands. These animals, nevertheless, responded readily to fright and other emotional disturbances with the usual symptoms of sympathetic excitation in the same manner as normal cats. Certainly, an outburst, through nervous influence, of epinephrin in such quantities as would be necessary to produce these symptoms would be impossible in these animals." Again, we fail to see the impropriety of quoting such a result, so long as it is true. We are glad that Doctor Cannon finds this result so obvious as not to be worth while mentioning. For it must then be obvious to him also that in these animals "the persistence of the emotional state after the exciting object has disappeared" cannot be explained as due to "continuance of adrenal secretion," whether "autogenous" or not.

Cannon seems to criticise our use of the term "denervated eye" for the eye after removal of the corresponding superior cervical ganglion, and says that he and de la Paz "tried the denervated eye method of testing for adrenal secretion but could not persuade themselves that an eye still innervated by the third cranial nerve was really denervated." It is surely unnecessary to state that when we use the term "denervated eye" in this sense we do so for convenience, following the practice of other writers, for instance, Elliott (15). We frequently speak of "the (denervated) eye reactions of Meltzer," or simply "the eye reactions," or of "the iris sensitized by previous removal of the superior cervical ganglion." Cannon himself uses the terms "excited" blood and "quiet" blood for convenience, and quite properly, although he will not maintain that they are literally correct. We did not imagine that any reader would suppose that we were not aware that the oculomotor nerve supplies the eye. We agree with Cannon that "the prompt dilatation of the pupil in a paroxysm of rage," in cats whose epinephrin output has been interfered with by removal of one adrenal and section of the nerves of the other has nothing to do with epinephrin, but is due to inhibition of the pupillo-constrictor mechanism. We have attributed the dilatation caused by stimulation of sensory nerves, including the paradoxical reaction, which can be obtained after removal of the adrenals (8), to the same mechanism. Long ago, it was stated by one of us (27) that sexual excitation caused very marked dilatation of the pupils, in dogs of both sexes, after division of both vago-sympathetic nerves (without removal of the superior cervical ganglion) by central inhibition of the constrictor pupillae through the oculomotor.

Apparently it is necessary to point out again, as has been done repeatedly before (1), (12), that when we employ the eye reactions to test for epinephrin, we eliminate factors which can affect the eye through the nervous system, by collecting the adrenal blood in a cava pocket and only releasing it, as far as possible, after these factors have ceased to act.

Cannon, on the basis of his experiments with the catheter method, concluded that emotional excitement, asphyxia and sensory stimulation increase the rate of output of epinephrin. Nicotine does the same, whereas urethane has no effect. Our own experiments with asphyxia and sensory stimulation, made by methods which permitted the rate of output of epinephrin to be estimated, did not support Cannon's conclusion, yielding no evidence of sensibly increased output. Such negative results must always yield to positive results obtained by a better method, and this has been emphasized by us. Cannon's catheter method, however, far from being a better method than those employed by us, is not a method by which the rate of ouput of epinephrin can be estimated at all. As a matter of fact, his papers do not contain a single estimate of the output of epinephrin before, during or after the action of the factors which he is studying. And it is clearly impossible that they should contain such data. The most that the method could yield would be the epinephrin concentration in the cava blood above the

adrenal level. Without knowing the amount of blood with this epinephrin concentration passing the point of collection per unit of time, the output of epinephrin could not be calculated. But even the epinephrin concentration was not determined by Cannon. He gives not one estimate of the concentration, or any information as to the minimum concentration which his strips or segments could have detected. In the absence of information of this kind it is impossible to deduce any conclusion from the few curves reproduced. What, for instance, was the concentration of epinephrin in the femoral vein blood after emotional excitement? If we knew whether it was 1:1,000,000 or 1:10,000,000 we might be able to check the probability of the statement by calculating the concentration which must have existed in the blood of the adrenal veins, and seeing whether anyone has ever observed so high a concentration. If the minimum concentration clearly detectable by the segments and the concentration actually found in the cava blood during asphyxia, etc., had been determined, there would be some possibility of deciding whether the observed changes in concentration could be due to alterations in the rate of blood flow in the cava without any change having occurred in the rate of output of epinephrin.

We have suggested that vasoconstriction, especially in the splanchnic area, which may be assumed to accompany the rise of blood pressure associated with stimulation of the central end of the sciatic or with asphyxia, will cause slowing of the blood flow in the inferior cava. Doctor Cannon seeks to invalidate this suggestion by the statement that an increased arterial blood pressure causes an increased blood flow through the *adrenals*. Had this argument not been repeated and emphasized in his paper we should have considered it a slip. Certainly the adrenal flow increases when the arterial pressure rises. But as the blood from the adrenals is only a small fraction (perhaps $\frac{1}{100}$ to $\frac{1}{200}$) of the blood passing along the inferior cava this, of course, has no sensible influence upon the rate of cava flow. Cannon further argues that when the arterial pressure is raised by splanchnic vasoconstriction the blood flow in the inferior cava may be increased. We had supposed it was universally admitted that vasoconstriction renders the passage of the blood through a vascular area more difficult, and that the arterial blood pressure rises for this very reason when an important area, like the splanchnic area, is constricted. We are not concerned here with compensatory reactions which may occur in other areas, leading, for example, as Edwards (16) has shown, to increased flow in the superior cava. It is precisely because less blood is passing through the vasoconstricted areas that more blood can pass through areas which are not constricted.

Vasomotor effects are not the only factors which may influence the rate of the blood flow in the inferior vena cava under the conditions of Cannon's experiments. The respiratory movements and, therefore, the intrathoracic pressure are affected by sensory stimulation, emotional disturbance and very grossly by

asphyxia. It is not possible to produce such changes without markedly affecting the pressure in the great veins. The region of the cava from which a sample of blood is assumed to be drawn off through the catheter is right up against the diaphragm. How can the control sample be obtained under the same conditions, except for the hypothetical stimulation of the adrenal secretory mechanism, as a sample taken when the animal is gasping in asphyxia? In asphyxia the right heart and great veins become engorged with blood. The blood in the cava must, therefore, be dammed back and if the adrenal goes on steadily secreting epinephrin the concentration in the upper segment of the cava must increase without any change having occurred in the rate of output. If the animal, in struggling or gasping, markedly increases the intraabdominal pressure the cava might be more or less obstructed by flattening of its walls upon the catheter, especially above its upper end.

There are still other ways in which the blood flow may be altered through effects produced upon the heart by the factors studied by Cannon. Yet in none of his papers has he indicated that there is any necessity to take into account possible changes in the rate of blood flow. It is the same with the question whether massage of the glands, particularly of the right adrenal, may not be produced by the catheter during the voluntary movements executed by the animal when frightened or the movements of the diaphragm in asphyxia. The right adrenal lies close against the cava, separated from the catheter by little more than the thin wall of the vein, and it is well known that massage causes liberation of epinephrin from the adrenals. All these possibilities ought to be controlled.

In referring to our negative results with the catheter method Cannon states, what could not be concluded from anything in his previous papers, that "the method is difficult and exacting, and that not until after some experience with it did it begin to yield us positive results." He rebuts our suggestion that some of his positive results might be due to a fortunate location of the eye of the catheter with reference to the orifices of the adrenal veins by saying "that it seems to have been made with disregard for the care exercised in making control observations under precisely the same conditions before and after stimulation." This is beside the point. There is no particular difficulty about inserting a fine catheter iuto the cava, so that in successive observations a string tied on the catheter is at the same level of the femoral vein, while the eye of the catheter is judged to be above the level of the adrenal veins. It is easy to turn the catheter so that a mark on its circumference always occupies the same position. But the exact relation of the eye of the catheter to the orifices of the adrenal veins or to the orifice of one of the veins, e.g., the right, since they do not enter the cava at the same level, can never be known till the abdomen is opened, if then, nor can it be assured that this relation will always remain precisely the same during such movements as the animal may make, even when securely tied down, during excitement caused by a barking dog or during stimulation of sensory nerves or asphyxia.

The few curves published by Cannon, are not at all convincing. In all the work with the catheter method he has contented himself with one or two comparative tests of the bloods. We have laid stress on the necessity of repeated comparison. His method of emptying the cylinder by sucking out one liquid

before the application of the next is also liable to introduce error, as we have previously pointed out (18) and illustrated by curves. Not only may the tracing be deformed at the critical moment, but the strip or segment is suddenly exposed and then suddenly immersed in liquid. He refers to a paper by three young medical men (17) as having confirmed his results on emotional excitement. But this paper contains not a single tracing or protocol, not one estimation of the epinephrin concentration in the blood or of the concentration which it would have been possible for the segments to detect. There is internal evidence that any reaction obtained could not have been due to epinephrin. For example, they state that when blood collected from the cava above the level of the adrenals, during the action of factors which increase epinephrin output, was allowed to stand for 25 minutes, it no longer caused inhibition of the rabbit's intestine segment, "and epinephrin is the only oxidizable substance in the body that produces inhibition of the intestine which can be oxidized in 25 minutes." Now, a sample of blood, known to contain epinephrin in amount sufficient to give a decided inhibition of the intestine, does not become ineffective merely by standing for 25 minutes. At one place it is stated that morphia prevents the epinephrin reaction from being given by the cava blood under the influence of Witte's peptone, and in another place that morphia distinctly diminishes the normal output of epinephrin, as tested in blood taken directly from the adrenal vein. In still another place the statement occurs that "substances with which we have experimented consisted of the toxins of gonococci, of streptococci, of staphylococci, of colon bacilli, of tetanus bacilli, of diphtheria, foreign proteids, indol and skatol, leucin, creatin, feces extract, strychnin, *morphia*, carbolic acid, Witte's peptone, sheep serum, ox serum, kitten serum. All of these have seemed to cause adrenal activation, and from their use 61 out of 66 consistent experiments can be reported . . . . Narcotics and anesthetics caused no increased output of adrenalin . . . . The various agents which caused increased epinephrin output, or which diminished it, are either neutral as to motion and fever or diminish them. . . . . There was no epinephrin reaction in any animal (subjected to intense emotion, rage and fear) in which bilateral division of the splanchnic nerves existed . . . . Of great significance is the fact that all agents that produced an increase of adrenalin output cause also a hyperchromatism of the brain followed by exhaustion. It mattered not whether this stimulating agent was a physical exertion in running, in fighting or in convulsions; or whether it was the emotion of fear, or of anger, or a reaction to anaphylaxis, to toxins, to indol, skatol, foreign proteids, or strychnin, in every case hyperchromatism and increase of adrenalin output went hand in hand." All this without tracings, without any sample of the great mass of experimental details which would be necessary to establish so many conclusions. If it is to be a matter of taking anybody's word for a physiological result, with all due respect to these writers, whom we know to be very competent men in their own subjects, we should much sooner take the word of an accomplished physiologist like Doctor Cannon himself.

In Cannon's paper on emotional stimulation the first figure shows that adrenal vein blood, obtained under ether, when substituted for inactive blood caused relaxation of the intestine strips, whereas blood from the renal vein did not give this effect. The most which could be deduced from this is that adrenal vein blood has a demonstrable content of epinephrin, a proposition which is generally

accepted. The second figure shows that when so-called excited blood (i.e., blood removed through the catheter from above the adrenal level during excitement) was substituted for Ringer's solution, the tone of the intestine strip was markedly increased. It remained increased for about 4½ minutes, and then the strip relaxed and the beats disappeared. Cannon interprets this extremely belated relaxation as an epinephrin reaction. This is contrary to all our experience. An epinephrin relaxation is produced immediately or not at all. A relaxation occurring 4 or 5 minutes after the application of a blood cannot be accepted as a positive reaction for epinephrin. A second application of the excited blood, this time replacing so-called "quiet" blood caused a prompt relaxation. The only conclusion which can be drawn from this figure is that two inconsistent observations with a given sample of blood on such an object as an intestine strip or segment cannot be accepted as evidence that the blood from the inferior cava, drawn during excitement, gave a positive epinephrin reaction. If an undoubted positive reaction had been obtained, this, as already pointed out, would yield no information as to whether the rate of output of epinephrin had been increased by the excitement or not.

The third figure professes to demonstrate that with the prolongation of excitement the effect on the epinephrin secretion goes on increasing. This is done by comparing a record of the strip in defibrinated blood drawn from the cava after 11 minutes excitement with a record in the *serum* of blood obtained after 15 minutes of excitement. In the defibrinated blood the strip retains the increase of tone caused by substituting the blood for Ringer's solution, for about 2 minutes, and then begins to relax. In the serum the strip relaxes more promptly. If the two samples of blood had the same concentration of epinephrin, the serum would necessarily give a stronger reaction than the blood since practically all the epinephrin is in the serum (19). After the initial increase of tone has lasted 2 or 3 minutes some relaxation occurs also with "quiet" serum, and the beats become slower. No data are given by which it would be possible to judge how great the difference in epinephrin concentration in the different samples was.

The fourth figure is intended to show that after removal of the adrenal glands, blood from the cava of an excited animal above the adrenal level gave no inhibition of the intestine strip when substituted for Ringer's solution. As there is no question that epinephrin exists in the upper cava blood, so long as the adrenals are intact, and must disappear when the adrenals are excised, nobody will dispute that a test object, if sensitive enough, will give a positive reaction in the first case and not in the second. In the legend of this figure, it is stated that "the strip later proved sensitive to adrenalin in blood in the ratio 1:1,000,000," and in the fifth figure tracings are given showing the inhibition produced by blood with 1:1,000,000, 1:2,000,000 and 1:3,000,000 adrenalin when substituted for "quiet" blood. The 1:3,000,000 adrenalin blood gives a reaction much smaller, and the 1:2,000,000 adrenalin blood a reaction probably smaller than those figured by Cannon as given by "excited" blood. He does not state specifically that these curves were obtained from strips used to test any of the "excited" bloods whose records are reproduced, and, as has been pointed out, it is unfortunate that he did not assay the concentrations of epinephrin which he believed these bloods to contain. But there would seem to be no point in reproducing tracings showing the inhibition of intestine strips given by such concentrations

of added adrenalin, unless they had a bearing upon the magnitude of the reactions given by the cava bloods. Redfield (20) who worked with Cannon's method, and partly under his direction, has stated that he succeeded in modifying the method so as to render it capable of detecting 1: 10,000,000 adrenalin. Redfield obtained such variable results in experiments, in which he endeavored to detect epinephrin in the blood (of the horned toad) during emotional excitement by means of the intestine reaction, that he says "no weight can be placed upon the experiments."

It seems pretty clear that in Cannon's hands the method was not very sensitive, and that such inhibitions as he figures, if due to epinephrin, must have corresponded to epinephrin concentrations in the "excited" cava blood of the order of magnitude of 1: 1,000,000 or 1: 2,000,000. Now, these concentrations are double the average concentrations found in adrenal vein blood, with the average normal arterial pressure under the conditions of our experiments, and would represent 100 to 200 times our average normal output. Cannon in his last paper (4) has stated that with sciatic stimulation he can increase the output to 5 to 25 times our "normal" output. But that apparently is nothing to what can be obtained when a cat is frightened by a dog. For us, such concentrations in the cava blood (corresponding perhaps to 1: 10,000 in the adrenal vein blood) are so improbable that we are unable to accept them on such evidence as Cannon has furnished. If anything like such concentrations of epinephrin could exist in the cava blood with ordinary rates of blood flow, it would be impossible for us to miss them with the method of assay which we employ.

In the sixth figure a tracing is given, showing that "active" blood applied to a strip caused relaxation after 2 minutes. The same blood, after oxygen was bubbled through it for 3 hours, when applied to a *fresh* strip is stated to have failed to cause relaxation. If the reactivity of the new strip was the same as that of the other (which was not controlled, but which must always be controlled if reliable results are to be obtained with such test objects) this would indicate that an oxidizable substance (epinephrin) was responsible for the original inhibition.

Cannon cites Redfield (20) as having "reported that in th - horned toad nervous excitement causes a contraction of the melanophores in the denervated skin, a reaction which does not occur after the removal of the adrenal glands." He adduces this as a proof that in this animal emotional excitement increases the epinephrin output. As a matter of fact, Redfield found that in the great majority of his experiments removal of the adrenals "does not check the contraction of the melanophores." Out of a large number of animals he only succeeded in finding two in which after adrenalectomy (involving the opening of the body cavity, removal of the gonads and a portion of the genital ducts and tying off the posterior cava) contraction of the melanophore pigment could not be obtained "when the mouth was stimulated by a weak faradic current." There is no proof that in these two exceptional cases it was the loss of the epinephrin output of the adrenals and not some other factors, such as the general deterioration of the animal caused by the operation, which was responsible for the negative result. However, his experiments with denervated skin and temporary ligation of the blood supply of portions of skin suggest strongly that epinephrin given off from the adrenals is a factor in contraction of the pigment. But we do not

find any proof that the effect is due to an increased epinephrin output. An experiment is quoted to show that blood from an excited horned toad causes a contraction of the melanophore pigment of an unexcited animal in the neighborhood of the point of injection, whereas blood obtained from an animal after destruction of the portion of the thoracic cord, whose integrity is essential to the production of contraction of the melanophore pigment through noxious stimuli, has no such effect. All that this experiment could indicate would be that the effect of the blood from the animal excited with intact cord was due to epinephrin. The experiment throws no light upon the question whether excitement can increase the epinephrin output. For if the injury to the thoracic cord prevents the contraction of the melanophore pigment associated with stimulation, by interfering with the ordinary output of epinephrin, the blood of the excited animal would necessarily contain more epinephrin than that of the control, without any increase in the rate of output having occurred in the former. We have shown (21), (22) that in mammals the upper part of the thoracic cord is intimately related to epinephrin secretion. The possibility must be considered that in this animal, on account of slower circulation, less active metabolism, and perhaps lower temperature of the blood and tissues, a greater concentration of epinephrin may exist in the general blood than in mammals. The great concentration found by Abel and Macht (23) in the secretion of the so-called parotid gland of a tropical toad suggests that care may be necessary in applying results obtained in such animals to mammals. The above remarks are in no way intended as a criticism of Redfield's paper, which seems to us a most interesting and suggestive piece of work.

In Cannon's paper on the effects of asphyxia, hyperpnoea and sensory stimulation on adrenal secretion there are three figures. Rabbit's intestine segments were employed for the tests. The first figure shows a marked effect of disturbance of the curve by sucking out the liquid. If any weight can be laid on a curve of this kind, the "normal" vena cava blood (i.e., from above the adrenal level in the absence of asphyxia) probably caused some inhibition and the cava blood from the same level after asphyxia, a greater inhibition. But how is it possible to determine whether the difference in epinephrin concentration between the two cava specimens is great or small?

The second figure purports to demonstrate the failure of hyperpnoea to increase adrenal secretion. "The chest was opened and the chest walls held apart while the lungs were inflated in rapid repetition by means of bellows. The air forced into the lungs was permitted to escape quickly through an opening in the trachea." The curve shows that when vena cava blood taken from above the adrenals with ordinary breathing is substituted for Ringer's solution, there is some increase of tone, but the beats continue. When vena cava blood drawn from above the adrenals after the application of hyperpnoea is substituted for the previous sample of cava blood no notable change occurs in the tracing. The conclusion is "that hyperpnoea, to a degree resulting in acapnia is not attended by increased adrenal secretion." This seems to us extremely probable, but what the tracing indicates is simply that no noteworthy change has occurred in the concentration of epinephrin in the cava blood, within the limits of sensitiveness of the segment. It is obvious that the conditions which in the asphyxia experiments may lead to slowing of the blood flow in the cava are absent here.

The chest being widely opened, changes in the intrathoracic pressure and in the movements of the diaphragm are eliminated. The situation as regards vasomotor and cardiac changes, especially splanchnic vasoconstriction, is likewise quite different. Since Cannon has here performed a considerable operation in an anesthetized animal, and since, according to him, the trauma and anesthesia in our experiments increase the "ordinary" output of epinephrin so much that it may be impossible for asphyxia or sensory stimulation to cause any further increase, the question would seem pertinent why he obtains in this experiment a totally negative reaction for epinephrin in the cava blood taken above the adrenal level. Our explanation is that the small adrenal output is too much diluted by indifferent blood in the cava to be easily detected.

The third figure in this paper purports to show that sensory stimulation increases adrenal secretion. What it does show is that when cava blood from above the adrenal level, obtained during sciatic stimulation, is substituted for cava blood obtained without sciatic stimulation, the writing point descends abruptly. A large part of the descent appears to be due simply to the fact that when the latter blood was sucked out of the cylinder the weight of the segment, coming on the lever, raised the point considerably. When the cylinder was again filled up with blood, the weight of the segment was nearly neutralized and the writing point necessarily descended. The true effect of the inhibition of the segment is, therefore, considerably exaggerated. All that can possibly be deduced from the tracing is that the epinephrin concentration in the sample collected during sciatic stimulation was greater than in the other. No assay of the concentration was made and, therefore, it is impossible to know what the difference was. It might have been a very moderate difference or a very great one. Inspection of such a curve can yield no information as to this. Also, it is not stated how many times the result figured was confirmed on the same samples of blood.

In his paper on the effect of nicotine on adrenal secretion Cannon has published three figures. The first shows that cava blood from above the adrenal level, secured before nicotine injection, when substituted for Ringer's solution caused an increase of tone of an intestine strip. When cava blood from the same level, obtained after injection of nicotine, was substituted for the other cava blood, there was a prompt and large diminution of tone. The conclusion is drawn that the adrenal secretion was increased by the nicotine. The most, however, which can be deduced from the tracing is that the concentration of epinephrin in the second specimen is greater than that in the first. If the blood flow is slowed, as would be the case after the doses of nicotine employed, an increased epinephrin concentration would be consistent, not only with an unchanged, but even with a diminished output. We have already pointed out (24) how completely Cannon, owing to the faulty method employed, has missed the details of the nicotine action on the epinephrin output, the most outstanding and most durable effect being a depression of the output.

It is stated in the text that the several specimens of blood were tested for their content of epinephrin. But not one tracing illustrating the assay is reproduced, nor is a single concentration given, although the statement is made that "the characteristic inhibition of the rhythmic contractions of the muscle, even when it has been for some time removed from the body, occurs at a dilution of adrenalin 1:2,000,000 in defibrinated blood." If it is implied that this is the order of

magnitude of the concentrations which cause inhibitions like that displayed in the figure under discussion, we can only repeat that it is in the highest degree improbable that such concentrations can be obtained in a fair sample of the cava blood above the adrenal level, unless there is an extreme slowing of the cava flow. Our own work on nicotine shows that at the time Cannon collected his samples the initial brief stimulating action of nicotine on the epinephrin output must have long since passed into the stage of depression. The effect of the first cava sample when substituted for Ringer's solution cannot, of course, be compared with that of the second cava sample when substituted for the first. To assay the two specimens they should be caused separately to displace the same sample of indifferent blood free from epinephrin. The displacement of one sample of epinephrin-containing blood by another sample of epinephrin-containing blood is not usually satisfactory for purposes of assay. For *a*, the first sample has already produced an epinephrin effect upon the test object which may render the inhibition, caused by the second sample when it displaces the first, different from what it would otherwise be. *b*, The epinephrin content of the first sample in contact with the test object, especially if left for a considerable time in contact with it, is diminished, so that the content of the second sample when now caused to displace the first may be exaggerated.

The second figure in Cannon's nicotine paper shows that the quantity of nicotine employed could not have accounted for the inhibition of the intestine strip produced by the cava blood after nicotine injection. This is unquestionably correct. The third figure demonstrates that cava blood obtained from an animal after removal of the adrenals and after administration of nicotine, does not cause an inhibition of the intestine strip. This is no doubt perfectly true, but it simply indicates that in the absence of the adrenals no epinephrin detectable by the method employed exists in the cava blood. It would be much more to the purpose to state what were the concentrations of epinephrin in the cava blood and the rates of cava blood flow before and after injection of nicotine. But the reader searches in vain for this essential information. That the intestine strip was inhibited by the addition of one drop of adrenalin 1: 1000 to a cylinder containing 2.5 cc. of blood (which would make a concentration of about 1: 35,000 or 1: 40,000) is no doubt correct. But what possible relation has a huge concentration like this to anything which can occur in the cava blood?

SUMMARY

1. The acceleration of the heart induced by asphyxia after division of the vagi and excision of the stellate ganglia cannot be taken as an index of increased epinephrin output from the adrenals, as assumed by Cannon. For it may be obtained and may not be diminished in amount after the adrenal veins have been ligated, after the adrenals have been completely isolated from the circulation, and after the adrenals have been excised.

2. Toward the end of an experiment, after repeated periods of asphyxia and when the general condition of the animal, including the

circulation, has deteriorated the reaction is apt to become much less marked or to fail altogether. An operation like adrenalectomy, when it accelerates the deterioration of the animal and of the heart by lowering the blood pressure decidedly, may sometimes seem to be responsible for the failure of the asphyxial acceleration. But there is evidence that this is not due to any specific effect of the adrenalectomy (loss of epinephrin output) but to the general effect of the operation. When asphyxia fails to cause a decided acceleration it is often seen that some slowing has occurred during the asphyxial period, succeeded by a quickening of the beat on resumption of respiration. It is clear that it will depend upon the state of the heart whether this subsequent acceleration will bring the heart back to, or nearly to, its original rate or cause it to pass considerably beyond the original rate. Whether an acceleration of some magnitude, as compared with the rate before asphyxia, can actually be counted on the trace is then more or less of an accident, depending upon whether the deteriorated heart comes through the period of asphyxia in such a condition as to permit it to attain a rate considerably in excess of the initial rate.

3. There is no good evidence that asphyxia causes an augmentation of the rate of epinephrin output by a direct action upon the cells of the adrenal medulla.

4. The acceleration caused by asphyxia, like the acceleration caused by stimulation of the central end of the sciatic, is not interfered with by opening the abdomen. There is no evidence that the operation necessary for collecting adrenal vein blood for direct assay of its epinephrin content would render it impossible to detect an increase in the epinephrin output, if this were caused by asphyxia or stimulation of sensory nerves.

5. No real evidence has been adduced that the epinephrin output, measured by those observers who have adopted the fundamentally correct method of collecting the blood and assaying it on suitable test objects, is an output artificially increased by anesthesia and trauma. On the contrary, the remarkably narrow range of the output in different animals, under different anesthetics and with the different operations strongly suggests that it is a physiological output already going on, not initiated and probably not much modified, but merely unveiled by the experimental procedures necessary for its measurement.

6. As a method of estimating changes in the rate of output of epinephrin from the adrenals, the catheter method is defective in principle. For at best all that could be measured by it would be changes in the

epinephrin concentration in the blood of the inferior cava above the level of the adrenals. Changes in the rate of flow of the blood are not taken account of. The quantity of epinephrin passing along the inferior cava to the heart per unit of time cannot be estimated, nor the changes, if any, produced in this quantity by the conditions studied. We have pointed out that in the cases in which Cannon claims to have obtained evidence of an increased output of epinephrin, all he can possibly have observed is an increased concentration in the cava blood, and that a slowing of the cava flow would cause an increase in the concentration, if no change whatever had occurred in the rate of output. We have suggested certain factors, associated with all the conditions studied by him, which might cause such changes in the cava flow as would tend to increase the epinephrin concentration, even in the absence of an increased rate of output. However, the complete lack of assays of the concentrations of epinephrin supposed to have been present, and of estimates of the concentration which could have been detected by each segment or strip employed, renders it impossible to determine to what extent positive reactions, when obtained, were actually due to epinephrin. It cannot be assumed that by sucking blood from a catheter opening into the cava immediately below the diaphragm, a region where the blood flow is necessarily less steady than anywhere else, owing to the influence of changes in the intrathoracic pressure, the same aliquot part of adrenal blood will be drawn off in successive samples without regard to the gross effects upon the respiration produced by such conditions, e.g., as asphyxia. Such mechanical changes may alter the proportion of adrenal blood to the much greater quantity of blood from other sources drawn off through the catheter. Our experience with the catheter method fully justifies the criticism of Richards and Wood (25) that the method is "highly faulty in that the blood from the suprarenals is diluted with that from all of the structures whose veins enter the cava below the suprarenals."

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THE EFFECT OF SPLENECTOMY UPON GROWTH IN THE YOUNG

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INTRODUCTION

With but few exceptions the removal of the spleen has been done on adult animals. Might it be that splenectomy in the young would exhibit some change not yet observed? It is known that removal of thyroid glands in young rabbits is followed by much more striking changes (experimental cretinism) than are manifested by removal of these glands in the adult animal.

LITERATURE

Wolferth (1) reports blood changes in rats splenectomized at thirty days of age, comparable to those changes in the blood of adult splenectomized rats. There is a slight to moderate anemia persisting for three or four weeks with recovery in a few months. Accompanying this is a slight leucocytosis and an increased resistance to hypotonic sodium chloride solutions and hemolytic agents.

Tachigara and Takayi (2) find that in general the effects of splenectomy in newborn dogs are the same as in adult animals. The most marked result is an increase in the number of red corpuscles. The younger the dog at the time of operation, the greater is this increase and the earlier does it appear. Their protocols likewise show that the splenectomized puppies operated from one day to two months make gains in weight that are nearly parallel to normal animals through periods of six to eight months. They also observed that the spleen of young animals weighs more in proportion to the body weight than it does in older animals. This is a factor that has been reported clinically by Gross (3) in a statistical study of the spleen.

On two male dogs from the same litter, one splenectomized and one a laparotomy control, operated by Doctor Luckhardt at four weeks of

age, the weight curves were found to differ. The splenectomized animal gained in weight but not in the same proportion as did the control. These dogs suggested a difference in metabolism as judged from the weight curves. These dogs were very kindly given to me by Doctor Luckhardt[1] at four weeks after operation for further observations. (Vide infra).

Removal of the spleen in adult animals presents results that differ, yet suggest that the spleen may have an influence in metabolism. Richet (4) reports in his metabolism experiments that splenectomized dogs must eat a much greater quantity to maintain the same nutrition as normal dogs. Spitta and Mayo (5) report an increase in weight following splenectomy. Paton (6) concluded that the spleen had no influence upon metabolism. Goldschmidt and Pearce (7) although differing from Paton on his blood studies, concluded that there was no effect upon metabolism, but observed that splenectomized dogs had a tendency to become obese. From observations in this laboratory upon adult splenectomized dogs it has been noticed that for a time following splenectomized dogs were emaciated but later became quite fat.

The possibility that the spleen assumed a greater functional importance in the young than in the adult animal as suggested by the proportionate differences in the weight of the organ at these two periods of life, as well as the contradictory results in metabolism of splenectomized dogs, led to this question: What effect has the removal of the spleen upon the metabolism of young animals as judged from the growth and weight curves?

METHODS

Operative procedure. In this work rats and rabbits have been the principal animals upon which the work has been done. Observations were made upon the dogs already mentioned and upon two series of kittens. Unfortunately distemper and accidents prevented further data on kittens and puppies.

The rats and rabbits were operated at fourteen days of age under ether anesthesia. Of the cats recorded, one series was operated at fourteen days and the other at seven weeks. It was found inadvisable to operate earlier than two weeks, on account of the extreme delicacy and softness of the young tissues.

[1]The work reported in this paper was done upon the suggestion of Dr. A. B. Luckhardt whose kindly interest and constant help has been most gratefully appreciated by me.

Removal of the spleen was made through an incision in the left rectus. Curved forceps were used in removing the spleen so that the incision might be small. In rats no ligatures were placed upon the splenic vessels but slight compression by forceps prevented any hemorrhage. In the rabbits, kittens and puppies silk ligatures were placed upon the splenic vessels. A frog board makes an excellent operating table for the rats.

That the operative procedure might be nearly comparable in the splenectomized and control animals, the controls were kept under ether the same length of time and the viscera were manipulated for a short time. In rats a few animals were observed which were unoperated controls. This was done as a check upon the effects of the operative procedure.

Diet. The diet of rats consisted of oats, bread, milk, carrots and, when obtainable, lettuce or cabbage. At intervals of two or three weeks raw meat was fed. The diet of rabbits was that of the rats save that bread, milk and meat were not fed.

The diet of the kittens was table scraps, bread and milk, and meat. The dogs were fed a diet similar to cats without the addition of milk.

Weighing. The animals were weighed just before operation and at weekly intervals prior to the time of feeding.

RESULTS

That individual variations might not lead to prejudiced or erroneous conclusions, composite weight curves were made of the rats and the rabbits.

Rats. In figure 1 is given the composite weight growth curve of five male operated controls and eight male splenectomized rats over a period of twenty-three weeks. Of these rats there is in table 1 a record of one typical set. In this table there is also a record of one unoperated control. This record is typical of all unoperated animals. The composite curves show that there is very little difference between the splenectomized and operated animal controls, the difference never exceeding 10 grams and this in favor of the splenectomized animals.

Figure 2 represents the composite weight curves of nine female splenectomized and eight female operated control rats over a period of twenty-three weeks. A typical set from this series is presented in table 2. As in figure 1, it may be seen that there is no appreciable difference in the weight curves. Here again the splenectomized ani-

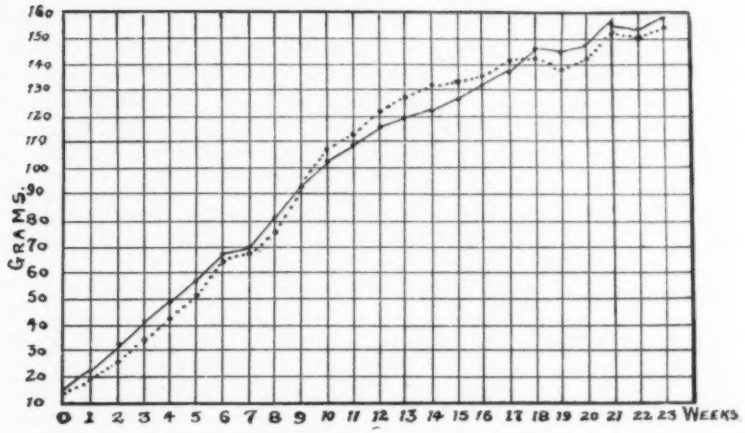

Fig. 1. Composite weight curve of male rats. Five operated controls———; eight splenectomized..... Operated at *0* when fourteen days of age and carried through a period of twenty-three weeks.

TABLE 1

Male rats

Three rats: no. 12, an unoperated control; no. 13, a splenectomized animal; no. 14, an operated control. The latter two animals were operated at fourteen days of age, and have their weights included in those of the composite curves of figure 1. The weights of the unoperated control were begun at fourteen days of age, and with those weights of the other two animals were carried through a period of twenty-four weeks.

	DATE OF OPERATION					
	2/4/19	2/12	2/18	2/25	3/4	3/11
Rat 12. Unoperated control	17	24	30	37	44	49
Rat 13. Splenectomized	17	24	31	45	58	68
Rat 14. Operated control	18	25	34.5	50	59	66

	3/18	3/25	4/1	4/8	4/15	4/22	4/29	5/6	5/13
Rat 12	56	62	70	82.5	98	109	117	120	127
Rat 13	74	85	92	112	137	144	147	154	160
Rat 14	72	83	92	110	130	137	145	153	162

	5/20	5/27	6/3	6/10	6/17	6/24	7/1	7/8	7/15
Rat 12	129	134	140	137	137	139	150	148	145
Rat 13	155	162	163	169	162	164	185	178	183
Rat 14	162	163	165	174	172	173	190	178	184

All weights are in grams.

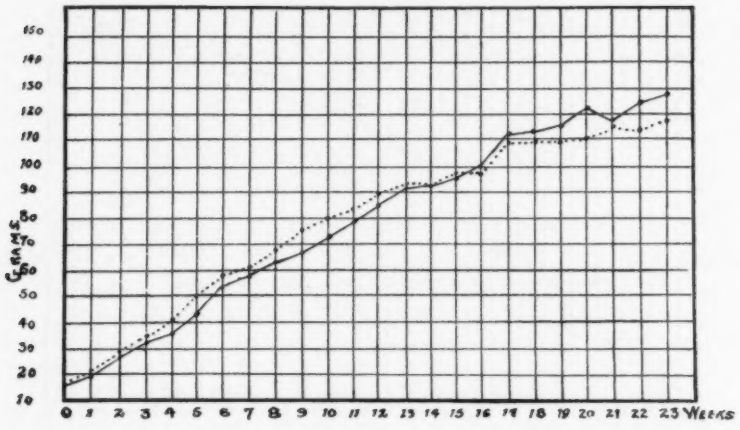

Fig. 2. Composite weight curve of female rats. Eight controls———, nine splenectomized..... Operated at *0* when fourteen days of age and carried through a period of twenty-three weeks.

TABLE 2

Female rats

Two rats: no. 27, an operated control; no. 30, a splenectomized animal. These animals were operated at fourteen days of age, have their weights included in the composite weight curves of figure 2, and were carried through a period of twenty-four weeks.

	DATE OF OPERATION					
	2/8/19	2/15	2/22	3/1	3/8	3/15
Rat 27. Operated control	11	18.5	22.5	26.5	29	33
Rat 30. Splenectomized	13	21	30	35	42	46

	3/22	3/29	4/5	4/12	4/19	4/26	5/3	5/10	5/17
Rat 27	39	45	51	58	60	67	72	74	72
Rat 30	60	67	76	94	87	92	98	97	99

	5/24	5/31	6/7	6/14	6/21	6/28	7/4	7/11	7/18
Rat 27	70	73	79	80	82	82	85	98	104
Rat 30	109	115	109	109	109	114	112	111	112

All weights are in grams.

mals have an average that exceeds the operated controls during the period of growth prior to maturity. It may be seen that the female rats gain in weight at a rate equivalent to the gains made by the male rats until they are about fifty days of age, at which time the male rats gain at a slightly faster rate. This is the normal manifestation shown by normal unoperated rats as observed by Donaldson, Dunn and Watson (8).

In two litters of rats weight records of one female and three male unoperated rats were made as a control upon operative procedure.

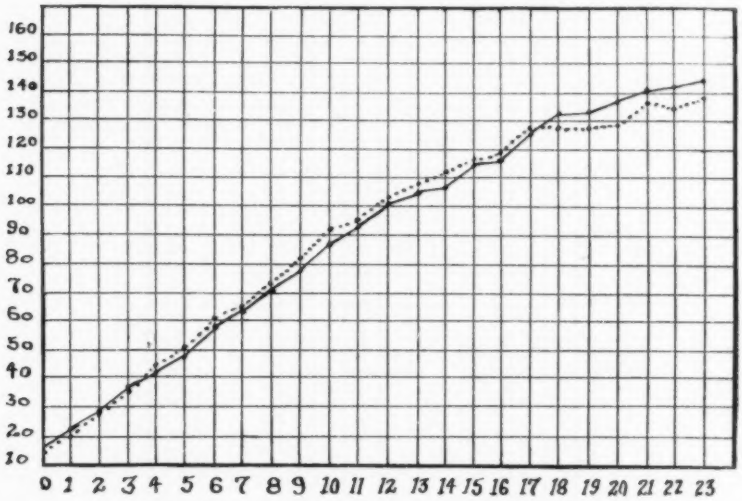

Fig. 3. Composite weight curve of all rats. Five male and eight female operated controls ———; eight male and nine female splenectomized..... Operated at *0* when fourteen days of age and carried through a period of twenty-three weeks.

The weights were below those of the operated animals, significant of the negligible influence that operation had upon growth of the young animals.

In figure 3 there is a composite weight growth curve of all splenectomized and operated control rats (male and female). In this graph it is evident that the weight curves are almost identical during the period of growth prior to maturity.

Rabbits. Figure 4 contains the composite weight growth curves of four splenectomized and four operated control male rabbits. In table 3 the weights of a typical set of animals from this group and also the weights of an unoperated control are recorded. In figure 5 there are the composite weight growth curves of four splenectomized and three operated control female rabbits. In table 4 a typical set from these series is recorded.

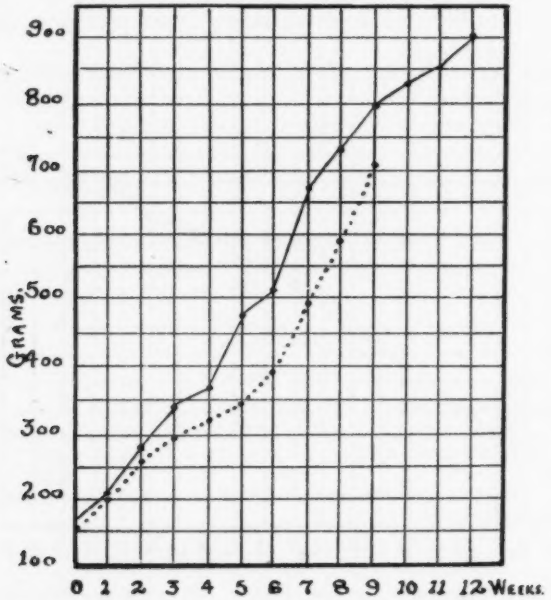

Fig. 4. Male rabbits. Composite curve of four operated controls ——— and four splenectomized rabbits..... Operated at *0* when fourteen days of age and carried through a period of twelve weeks.

It may be noticed that the general average of the male operated controls is above that of the splenectomized male rabbits and that the average of the female splenectomized rabbits is above that of the female operated controls. The differences are too small, considering the difference in their weights at the beginning of the records, to attempt an explanation through any effect the spleen may have upon metabolism.

TABLE 3

Male rabbits

Three rabbits: no. 5, a splenectomized animal; no. 6, an operated control, and no. 7, an unoperated control. The operated animals were fourteen days of age at time of operation and have their weights included in the composite weight curves of figure 4. The unoperated control was weighed at fourteen days of age, and thereafter weights were taken each week as were those of the operated animals.

	DATE OF OPERATION						
	1/24-19	1/28	2/6	2/12	2/19	2/22	3/5
Rabbit 5. Splenectomized.....	144	156	227	300	290	340	360
Rabbit 6. Operated control.....	174	195	284	310	357	352	390
Rabbit 7. Unoperated control...	145	202	292	365	359	342	375

All weights are in grams.

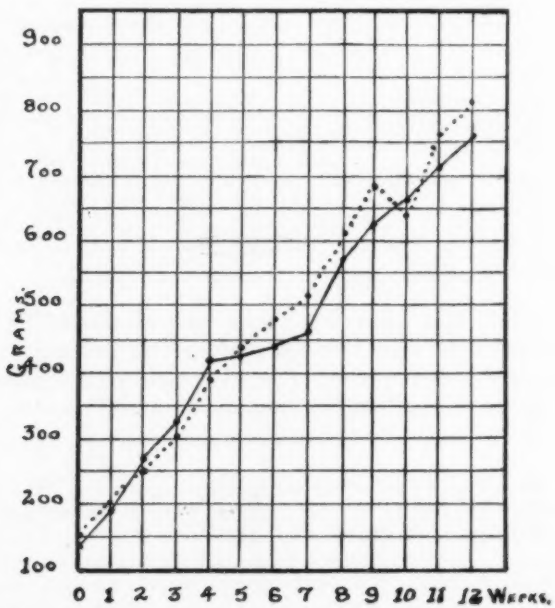

Fig. 5. Female rabbits. Composite curves of three operated controls ——— and four splenectomized..... Operated at *0* when fourteen days of age and carried through a period of twelve weeks.

In figure 6 are given the composite weight growth curves of all the male and female rabbits. Here it is evident that there is practically no difference in the growth curves of the splenectomized and operated control animals, especially when it is taken into consideration that the average of the operated controls was 20 grams above the weight of the splenectomized animals at the beginning of the records.

Kittens. In figure 7 there is the record of the weights, over a period of nine weeks, of kittens operated at fourteen days of age. Their record although irregular has the same general tendency as with the rats

TABLE 4

Female rabbits

Two rabbits: no. 11, a splenectomized animal; no. 9, an operated control. These animals were operated at fourteen days of age, have their weights included in the composite weight curves of figure 5, and were weighed through a period of thirteen weeks.

	DATE OF OPERATION					
	3/9/19	3/15	3/22	3/29	4/5	4/12
Rabbit 11. Splenectomized	155	182	239	319	382	512
Rabbit 9. Operated control	136	163	224	247	299	330
	4/19	4/26	5/3	5/10	5/17	5/24	5/31
Rabbit 11	506	602	614	634	507	672	725
Rabbit 9	307	346	425	420	443	465	470

All weights are in grams.

and the rabbits, that there is a similar gain manifested by splenectomized animals as with the operated control animals. In other kittens operated at seven weeks of age the same relative curves were observed in the splenectomized animals as in the operated control kittens.[2]

[2] Of the first litter of two kittens the splenectomized kitten did not gain in weight as did its control mate. It died about the tenth week weighing 260 grams. Its mate at that time weighed 800 grams. At the time of operation each weighed about 150 grams. Necropsy revealed congenital (?) absence of the thymus of the splenectomized animal. It is worthy of note that the splenectomized animal possessed adrenal glands which were twice as large and weighed almost twice as much as the adrenal of the control animal which was sacrificed for examination on the day of death of the other.—A. B. L.

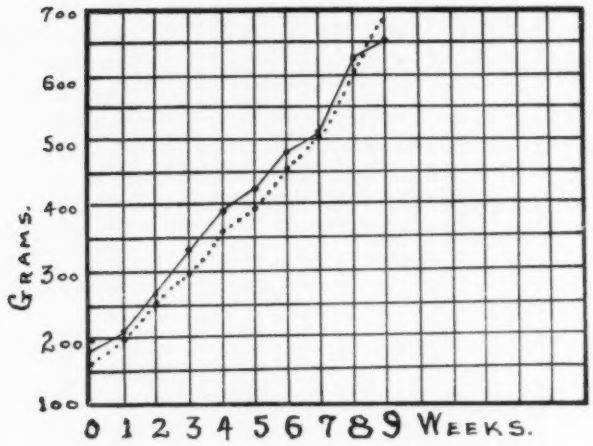

Fig. 6. Composite curves of all rabbits. Four male and three female controls ———; four male and four female splenectomized.....

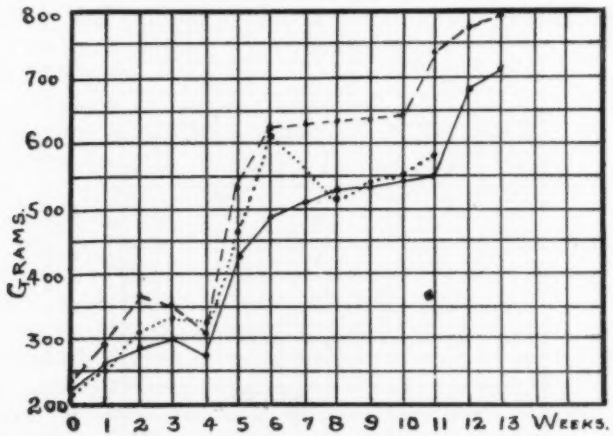

Fig. 7. Weight curve of kittens. One male control ———; one male splenectomy ———; one female splenectomy..... Operated at *0* when fourteen days of age and carried through a period of thirteen weeks.

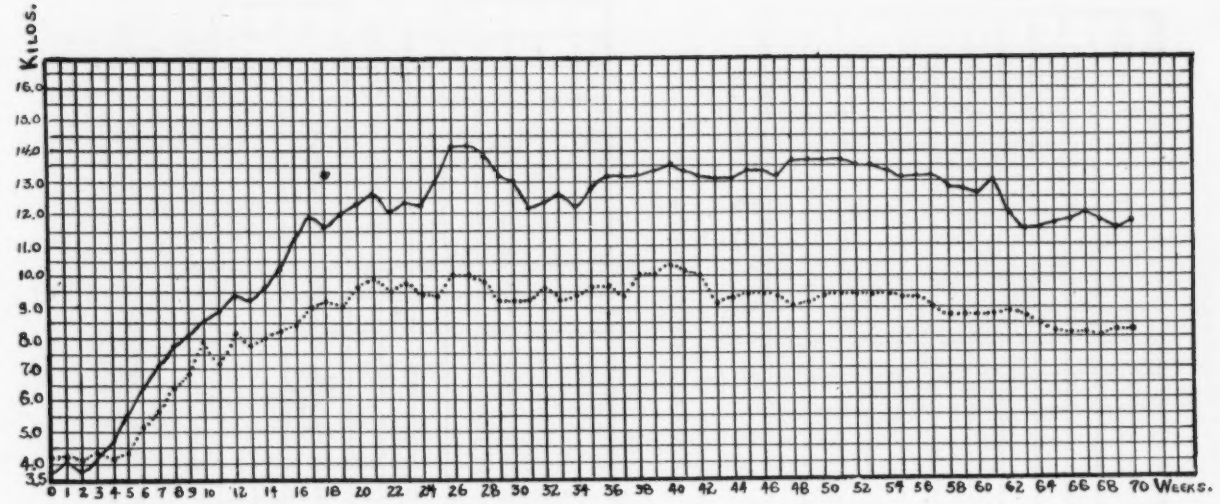

Fig. 8. Weight curve of dogs. Male operated control ——; male splenectomized..... Operated at *0* when twenty-eight days of age and carried through a period of seventy weeks.

Puppies. Figure 8 of the dogs over a period of seventy weeks after operation at four weeks of age shows a marked difference in weight, but failure of others to withstand distemper after operation, and in light of former work on puppies (Tachigara and Takayi) there is insufficient data upon which any conclusions might be drawn in connection with growth. During the month following the operation these dogs had severe attacks of distemper. In addition to distemper the splenectomized animal had severe gastro-intestinal disturbances as was evidenced by vomiting and diarrhea. These factors may have had an inhibitory effect upon growth.

OTHER OBSERVED EFFECTS OF SPLENECTOMY IN THE YOUNG

Blood changes

Dogs. As this problem did not primarily deal with the blood picture, complete records have not been made. Blood counts were made on the dogs seven months after splenectomy. The splenectomized animal showed a general leucocytosis of 23,000 and moderate anemia (6,000,000 erythrocytes) compared to the counts made on the blood of control dog, which averaged 11,000 leucocytes and 8,600,000 erythrocytes. The same pipettes were used in making the counts on the two animals. The differential counts of blood cells from both animals gave percentages of cell types nearly equal.

a. Resistance to hypotonic sodium chloride solutions. Dogs. The blood corpuscles of splenectomized animals were found to be more resistant to hypotonic sodium chloride solutions than were the corpuscles of normal dogs.

Following the method of Karsner and Pearce (10) 0.1 cc. of corpuscular mass was placed in each of a series of tubes containing 3.0 cc. of sodium chloride solutions varying from 0.1 per cent to 0.5 per cent by differences of 0.025 per cent. Readings were made at the time the corpuscles were mixed with the salt solutions and eighteen hours later, having been kept in a refrigerator near ice. Corpuscles from two male dogs, ten weeks after being splenectomized at four months of age, gave 100 per cent hemolysis at 0.4 per cent and all solutions of lower percentage, with only a trace at 0.5 per cent. From a female dog, nine months after splenectomy at ten weeks of age, and from splenectomized male of figure 8, there was practically no hemolysis in solutions above 0.25 per cent, with no trace of hemolysis above 0.4 per cent. In four animals unoperated and varying in age from ten months to eigh-

teen months of age, there was complete hemolysis at 0.35 per cent and below, with no trace of hemolysis above 0.45 per cent. These results are in keeping with the observations made by Karsner and Pearce. They report similar results as having been observed by Bottazzi, Banti, Pugliese and Luzzati, and others.

TABLE 5

In this table there is a record of the coagulation time of the blood from three dogs. No. 1 was a male operated control, operated at four weeks of age and seventy-five weeks of age at this time. No. 2 was a male dog, splenectomized at four weeks of age, now seventy-five weeks of age. No. 3 was a female dog, splenectomized at ten weeks of age and now fifty weeks of age.

	SAMPLES	BLOOD DRAWN	FIRST EVIDENCE OF COAGULATION	FIRM CLOT	TIME OF COAGULATION
					minutes
Dog 1	1	9:38	9:42	9:45	7.0
	2	9:38	9:42	9:45.5	7.5
	3	9:38	9:42.25	9:46	8.0
	4	9:38	9:43	9:46	8.0
	5	9:38	9:43	9:46	8.0
	Average time				7.7
Dog 2	1	10:09	*	10:10	1.0
	2	10:09	*	10:10	1.0
	3	10:09	*	10:10	1.0
	4	10:09	*	10:10	1.0
	5	10.09	*	10:10	1.0
	Average time less than 1′				1.0
Dog 3	1	2:55	2:56	2:57.5	2.5
	2	2:55	2:56	2:57	2.0
	3	2:55	2:56	2:58	3.0
	4	2:55	2:56	2:58	3.0
	5	2:55	2:56	2:58	3.0
	Average time				2.7

Rabbits. Corpuscles from a female rabbit, ten months after splenectomy at two weeks of age, were found to be no more resistant to hypotonic sodium chloride solutions than were the corpuscles of an operated control male rabbit of the same age.

b. Coagulation time. Dogs. In drawing blood from the ear and saphenous veins, the blood from splenectomized animals clotted much quicker than that from operated controls and normal animals. In table 5 the decided difference may be seen between the time of coagulation of blood in an animal splenectomized early, one splenectomized later, and the blood from a normal operated control animal.

Upon centrifuged blood of dogs of figure 8, it was found that corpuscles were decreased and serum increased in the splenectomized animal. The percentages were: corpuscles, 31.12 per cent; serum, 68.87 per cent; while in the control there was 58.66 per cent corpuscles and 41.33 per cent serum.

Rats. In seven male controls, seven male splenectomized, three female controls and four female splenectomized rats, there was no detectable difference in the time of the coagulation of their blood. Two samples of 1 cc. each were taken from the tail and the heart. Coagulation of the blood occurred in one minute.

Rabbits. In rabbits the coagulation time was determined of the same two as are recorded under "resistance to hypotonic solutions." The time of coagulation in five tests was found to be the same for each animal. These two animals lived longer than those that are recorded in the graphs.

Appearance and temperament

Rats. No difference in appearance and temperament could be observed between the splenectomized animals, the operated controls and the unoperated controls. Other people in being allowed to examine the rats and questioned as to what difference they might observe, could not differentiate between splenectomized and control animals.

Kittens. In the kittens a difference in temperament but not in appearance was observed. The splenectomized animals were more active and pugnacious, but as cats are very emotional animals no particular importance was attached to this difference.

Dogs. In appearance the splenectomized animal was emaciated, although fed an equal amount of food as the control. In temperament and activity both dogs responded alike. The splenectomized animal contracted mange at two different times while the operated control was resistant, while living under the same conditions.

Fertility

Rats. Two female splenectomized rats gave birth to litters of rats. The male in each instance had been completely splenectomized. The mothers cared for their offspring in a manner comparable to normal rat mothers.

Rabbits. A female splenectomized rabbit gave birth to a litter of rabbits. The male was an operated control. The young rabbits were well cared for.

Dogs. The male splenectomized dog of figure 8 was known to have copulated with a female animal which had been splenectomized at ten weeks of age. Two weeks later the mammary glands of the female became noticeably enlarged simultaneously with which there was a fall in the leucocytic count from 25,000 to 18,000. Two weeks later the swelling of the mammary glands disappeared and the leucocytic count rose to 29,000. No pups were ever delivered. Histological examination of the male's testes showed a very active spermatogenesis.

Macroscopical and microscopical examination of tissues

Rats. Macroscopic examination of the rats revealed no changes of especial note save that in most of the splenectomized animals there was red bone marrow in the femur. There were several of the control animals that showed traces of red in the femur, so that this finding was not limited to the femur of the splenectomized animals alone. In three of the splenectomized animals small bits of splenic tissue were found. It was interesting to note that these animals did not grow as rapidly as did the animals that had complete removal of the spleen or were only control animals.

Microscopically the only difference that was detected was in the bone marrow. The bone marrow of the splenectomized rat was very rich in cellular elements composed principally of myelocytes, erthrocytes, mononuclear cells and a few multinucleated cells. The fat content composed about one-third of the marrow. The bone marrow from the operated control was very cellular, composed of cells very similar to those of the splenectomized animal. Two-thirds of the marrow was composed of fat.

Rabbits. Macroscopic examination revealed a liver in the splenectomized female that weighed more than in the male control although the control weighed more than the splenectomized animal. The

difference in body weight was the normal difference between a male and a female. The marrow of the femur of the splenectomized animal was streaked with red, while it was distinctly yellow in the control.

Microscopically the liver of the splenectomized animal was marked with many Kupffer cells containing erythrocytes in different stages of disintegration. The bone marrow was composed of one-third fat. The remaining portion consists of many multinucleated lymphoid cells, myeloblasts, mononuclear lymphoid cells, nucleated erythrocytes, erythrocytes and a few endothelial and giant cells. The mononucleated lymphoid cells appear very much like splenic cells. The liver of the operated control was moderately cirrhosed (probably explaining difference in weight), and contained a few Kupffer cells in which there were erythrocytes. The bone marrow was composed of nearly all fat, with a few areas near the periphery containing a few lymphoid cells and myelocytes.

Kittens. Macroscopic examination revealed no observable changes. The bone marrow was distinctly yellow in the femur of the splenectomized animal as it was also in the control. No microscopic examination was made.

Dogs. Splenectomized animal: Macroscopically the most marked changes appeared in the lymph glands, bone marrow and in the weight of the liver which was 30.9 per cent of the body weight. The mesenteric and retroperitoneal lymph glands were distinctly red in color. The inguinal, axillary and popliteal glands were enlarged and white and firm in consistency. The intestine gave evidence of only *one* extremely atrophied Peyer's patch. The bone marrow was distinctly red in color and fairly firm in consistency.

Microscopically the liver contained many Kupffer cells in which there were many erythrocytes and a few leucocytes. The atrophied Peyer's patch was the seat of very little lymphoid tissue in the submucous layer. The mesenteric lymph glands in the periphery contained regularly arranged whorls of lymphoid tissue. In the medullary portion there were smaller and less regular whorls of the same tissue. There were marked sinuses in this portion containing few lymphoid cells, and many endothelial cells packed with erythrocytes, and a few leucocytes. There were also many multinucleated lymphoid cells present in these sinuses. The inguinal lymph glands were composed of more irregular lymphoid tissue than that in the mesenteric glands. There were several endothelial cells containing erythrocytes and there were many more multinuclear lymphoid cells present than were in the mesenteric

glands. The bone marrow was densely packed with cellular material interposed with small amounts of fat. The cellular element was composed of erythroblasts, erythrocytes, myeloblasts, mono- and multi-nucleated lymphoid cells and a few giant cells. The testes showed a very active spermatogenesis.

Operated control: The lymph glands were not enlarged, the bone marrow was distinctly yellow in color and the liver composed 23.2 per cent of the body weight.

Microscopically the lymph glands were rather compact and consisted of mononucleated lymphoid cells. The liver contained Kupffer cells but not to the marked degree that they were found in the liver of the splenectomized animal. The bone marrow was composed of a great amount of fat with very few mononuclear cells and myeloblasts interposed. The testes appeared very similar to those of the splenectomized animal both in structure and in showing a very active spermatogenesis.

DISCUSSION

In this work there are results from rats and rabbits in numbers large enough to permit of conclusion of the effect of splenectomy upon growth. It would not be justifiable in drawing conclusions upon the two series of kittens, although their weight curves are similar to the curves of the rats and the rabbits. In the dogs there were early influences of distemper and gastro-intestinal disturbances that present a factor that was not ruled out by other animals. However from the carefully prepared protocols of Tachigara and Takayi it is suggested that puppies would present weight curves similar to the curves of the rats and rabbits. Mann, in speaking of rabbits, dogs and kids, says: "Our results show that some animals from which the spleen has been removed quite early in life develop in the same fashion as the unoperated controls; furthermore, no definite specific change in the development due to the loss of the spleen was observed in any of the animals" (10).

The resistance shown by the corpuscles against hypotonic sodium chloride solutions is found to be true of animals operated while young as well as adult splenectomized animals. This phenomenon suggests that the spleen may have the function of preparing the erythrocytes for destruction.

The quickened coagulation time of the blood from splenectomized dogs may be a protective means against loss of blood by hemorrhage

during the time of anemia. The results would suggest that the earlier an animal is splenectomized, the more rapid is the time of coagulation. It may be due to the general leucocytosis or to an increased amount of fibrinogen which may account for the large amount of fibrin secured when the blood from splenectomized dogs is defibrinated. Increased fibrinogen may be due to a greater production by the liver in the absence of the spleen. No other mention of coagulation time of blood from splenectomized animals was found in a review of the literature save by T. G. Orr (11) working on rabbits. He reports that in twelve splenectomized rabbits there was no change in coagulation time as compared to normal rabbits. Coagulation time of the blood from rats and rabbits was found to yield no important differences in our own series.

In activity and temperament no difference was observed in rats, rabbits or dogs, but a slight difference in kittens was observed. This may be due to some individual variation rather than due to any effect following removal of the spleen.

Loss of fertility was suggested by the female splenectomized dog's failure in bearing any pups; but the active spermatogenesis of the male's testes and the swelling of the mammary glands at an early stage after copulation indicates that some other factor during early pregnancy may have had an inhibitory influence upon the development of any embryos. In rabbits it was clear that the female was not sterile. In rats it was evident that neither male nor female was sterile.

Histological examination indicates that the function of the spleen is in some way connected with the blood. This is evidenced by large hemolymph glands especially in the mesenteric region, which suggests that if it is due to a compensatory hyperplasia following splenectomy, it is important that such a function should be produced in the portal circulation. The bone marrow changes were rather striking. It may be that in disturbing a portion of the blood apparatus there is a compensatory functioning of other portions. The bone marrow through the different types of cells present, indicates that there is a process of erythrogenesis and leucogenesis taking place, while an increase of the Kupffer cells and endothelial cells of lymph glands indicates a process of blood destruction.

Changes in lymph glands and in the bone marrow similar to the above have been described by Pearce and Pepper (12) and by A. S. Warthin (13).

CONCLUSIONS

1. The spleen is not essential to the life of young rats, rabbits, kittens and puppies.

2. Splenectomy has a negligible influence upon growth of rats and rabbits. It is suggested from the weight curves of the kittens that the same is true of these animals. Results of Tachigara and Takayi and Mann would indicate the same as being true of dogs.

3. Operative procedure has no debilitating effect upon the growth of young rats.

4. In dogs splenectomized while young there is a hastening of the coagulation time of blood. The younger the animals have their spleens removed, the more rapid is the coagulation time of the blood.

5. Corpuscles of young splenectomized dogs have the same property of more strongly resisting hypotonic sodium chloride solutions, than control and operated control animals, as do animals splenectomized after having reached maturity.

6. Accompanying the removal of the spleen there is an increase of Kupffer cells in the liver and changes in lymph glands and bone marrow that indicate a compensatory function on the part of these tissues in supplying a missing factor in blood formation and destruction.

7. Male dogs, male and female rats, and female rabbits are not rendered sterile through the removal of the spleen while these animals are young.

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THE IMPORTANCE OF VAGAL AND SPLANCHNIC AFFERENT IMPULSES ON THE ONSET AND COURSE OF TETANIA PARATHYROPRIVA

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INTRODUCTION

The object of this work was to determine, if possible, whether the afferent impulses which reach the central nervous system through the vagi and the splanchnic nerves have any influence on the onset and course of parathyroid tetany. That these might be of some importance was suggested to us by the great frequency with which tetany and gastro-intestinal disorders are associated clinically. Thus Osler (1) in quoting Frankl-Hochwart on the etiology of tetany in adults describes the "tetany of gastric and intestinal disorders, as dyspepsia, gastrectasis, diarrhoea, and helminthiasis," and again says that in children "tetany bears a definite relation to gastro-intestinal disorders." Paton, Findlay and Watson (2) mention this relationship in their classic on the parathyroids but do not seem to attach any significance to it.

McCann (3) attributes the tetany to the alkalosis resulting from the stagnation of the acid secretion in the stomach but Osler (op. cit.) says that "the type associated with dilatation of the stomach is rare, only thirty instances being reported" and hence this would not explain the great majority of cases. Ivy (4), from work done in this laboratory, reports that in eight dogs with duodenal fistulas which allowed everything the dogs drank together with the gastric juice to drain to the outside, only one developed tetany. This animal was advanced in pregnancy and the tetany he attributed to eclampsia of pregnancy. However Rodman (5), Bene (6) and others consider gastric ulcer and dilatation to be more closely associated with tetany.

Oppenheim (7) in discussing tetany says:

> Adults who suffer from gastro-intestinal affections are sometimes affected. . . . . It has not been definitely decided whether the toxic agent is the cause of the spasm or whether the spasm has a reflex origin. The fact that it has occa-

sionally occurred directly after the use of a stomach pump or the passing of a tube or after profuse vomiting seems to point to a reflex origin, and the observation—which cannot be implicitly relied upon—that the spasm has disappeared after the evacuation of entozoa confirms this theory. It is remarkable that Gerhardt was able in a case of tetany with dilatation of the stomach to bring on the spasm by slight percussion of the stomach.

In this laboratory it has been frequently observed that an attack of tetany may be precipitated in parathyroidectomized animals by the passage of a stomach tube, other sensory stimuli also having the same effect.

Carlson and Jacobson (8) and later Friedman (9) also recorded the great frequency with which hyperemia, hemorrhages and ulcers of the stomach and intestinal mucosa are found in the post-mortem examination of parathyroidectomized animals and were reminded of the relationship between gastro-intestinal disorders and tetany in man, stating further that "it is not known to what extent this tetany is due to toxic products absorbed into the blood or to irritation of afferent nerve endings, but in late years numerous attempts have been made to bring all types of clinical tetany within the category of parathyroid tetany." A priori we would reason that if irritation of the afferent nerve endings in the gastro-intestinal tract is ever of importance in tetany, it should be in that of parathyroid origin for in this condition we have not only these alimentary disorders but also a hyper-irritable condition of both the central nervous system and the peripheral nerves, as has been shown most completely by Paton, Findlay and Watson (op. cit.). Before undertaking this work it was thought that examination of the literature upon the effect of section of the cord at different levels might throw some light upon the subject but the work of Biedl (11), Falta and Rudinger (11), Paton, Findlay and Watson (2), Mustard (12), Carlson and Jacobson (8), and of Luckhardt, Sherman and Serbin (13), is at such variance that no conclusions on this point can be drawn.

EXPERIMENTAL METHODS

Two methods of procedure have been followed in these experiments. The first consisted in the determination of the effects of double vagotomy and splanchnectomy on the onset and course of parathyroid tetany and the second in the determination of the effect of gastro-intestinal irritation by means of a drastic, i.e., croton oil, upon the onset and course of parathyroid tetany. It was recognized of course that this artificial irritation is not strictly analogous to the normal irritative condition. Dogs were used exclusively in the work.

The technique of the first method was divided into three stages. In the first operation the thorax was entered on the left side between the sixth and the seventh ribs. Here the thoracic sympathetic trunk was picked up and all the rami communicantes torn loose in the lower part of the thorax, care being taken to destroy all threads of nerve tissue running from the rami communicantes to the left splanchnic. This nerve trunk was then grasped with the fingers and jerked out from the diaphragm. After thus getting rid of the left splanchnic, the esophagus was picked up and the vagi, which are found lying on each side at this level, were sectioned. Care was taken here to make sure that all of the vagus fibers were cut. The closing of the thorax completed the operation. Ether anesthesia alone was used, no morphine being employed because of its depressing effect upon the respiratory center. Artificial respiration was unnecessary unless the thorax was allowed to remain open for too long a time, in which case asphyxiation resulted.

After a week or so when this wound was found to be well healed and the animal in good condition, the second operation was performed under morphine-ether anesthesia. This consisted in entering the abdominal cavity through a right lumbar incision and picking up the right splanchnic just after it pierces the diaphragm. After dissecting this down to the point where it branches out to enter the coeliac plexus, the nerve and ganglion were removed and the abdomen closed. This operation is not so depressing as has been supposed, the dogs appearing lively and in good condition within a few hours after the operation and usually live in the best of health, apparently suffering no ill effects at all. The success of the two operations was determined at the subsequent post-mortem examination.

After a week or ten days had again been allowed to elapse, a complete thyro-parathyroidectomy was performed, the glands being removed grossly with as much of the surrounding loose tissue as possible in order to avoid leaving behind any accessory parathyroid tissue in that region. The animals were kept under the same conditions as before the operation and on the same diet (bread and meat). The electrical excitability of the femoral nerve was determined before the final operation and the change afterwards followed although it was expected from the work of Paton, Findlay and Watson that double vagotomy and splanchnectomy would have no effect upon the change in the electrical excitability of the peripheral nerves even though it did have an effect upon the tetany.

The technique of the second method consisted also in complete thyro-parathyroidectomy in the manner just described, followed by the

daily administration of croton oil in varying amounts. The U. S. P. dose of this drug is 2 minims and hence the 2 to 4 minims given daily to the dogs produced very severe irritation. The animals were kept under the same conditions as those of the first series and in most cases the electrical excitability was followed here also.

RESULTS

The animals developed distemper so frequently that this proved to be quite a troublesome factor, especially in the first series. In a large proportion of the cases autopsy showed a terminal infection with pneumonia. It is quite conceivable that this depressing condition might alter the course of the tetany somewhat but its effect was probably similar in the two series.

Effect of double vagotomy and splanchnectomy. Table 1 gives a summary of the results obtained in the first series of experiments. Nine of the sixteen animals developed tetany, five showed depression only and two are still living. The failure of these last two animals, dogs 48 and 52, to show any symptom is probably to be attributed to the presence of accessory parathyroid tissue. Excluding these from consideration, nine out of fourteen animals, or 64 per cent, developed tetany and five showed depression only. In the series of thyro-parathyroidectomies on dogs reported by Paton and Findlay, twenty-six out of forty-two, or 61 per cent, showed tetany. The severity of the tetanic attacks seemed to be as great in these dogs as in normal parathyroidectomized dogs and even greater than in the dogs of series 2. The protocol of the dog showing the most pronounced tetany in this series is given in table 3. It will be noticed that this dog vomited frequently, which point is also of interest since it points to direct central stimulation as the cause of the vomiting.

Effect of artificial gastro-intestinal irritation. Table 2 gives a summary of the results obtained in the second series of experiments. Here eleven out of fourteen dogs, or 78 per cent, showed tetany and three depression only. Although this is a somewhat higher percentage than that of series 1, it is scarcely of significance, especially in view of the fact that the severity of the tetany in these animals did not seem to us to be quite so great nor the attacks so prolonged as in those of the first series. The protocol of the animal which showed the greatest tetany in this group is given in table 4. Although this animal showed pneumonia at autopsy while that of the first protocol did not, it lived longer in spite of the pneumonia and hence the comparison between the two is a rather fair one and illustrates the similarity between the two series.

TABLE 1

Effect of double vagotomy and splanchnectomy on parathyroid tetany

DOG	FIRST OPER.	SECOND OPER.	THIRD OPER.	ONSET	NO. ATT.	SEVER.	DEP.	E. E.	DUR. EXP.	REMARKS	AUTOPSY
					hours				*days*		
7	4/8	4/19	4/23	125	2	++	+	+	6		Pneumonia; br. rt. spl. intact
30	4/30	5/31	6/11	0	0	0	+	+	1¾		Pneumonia; both oper. complete
32	5/1	5/15	6/11	67	2	++	+++	++	11½		Pneumonia; br. lft. spl. intact
33	5/1	5/14	6/11	35	3	+++	+++	++	8¼	Groans continuously; enophoria	Slight pneumonia; br. left spl. intact; hem. in intestines
36	5/5	5/9	6/10	26	2	++	+	+	2¼		Hyper. gastric mucosa, both oper. complete
38	5/10	5/13	6/10	41½	2	++	+	++	2		Both oper. complete; hem. in intestines
39	5/10	5/24	6/8	15	2	++	+	+	1½	Advanced pregnancy	Rt. spl. ima intact
40	5/13	6/4	6/10	0	0	0	+++	++	9	Slight tremors only	Pneumonia; thread of rt. spl. intact; hem. in intestines
44	5/17	5/23	6/14	0	0	0	+++	++	5¼	Vomits, distemper	Pneumonia; both oper. complete
46	5/19	5/23	6/10	60	4	+++	+	++	11		Pneumonia; both oper. complete; hem. in duodenum
47	5/20	5/27	6/10	0	0	0	+++	+	3	Very sick, distemper	Pneumonia; both oper. complete; hyper. gastric mucosa
48	5/24	5/31	6/16	0	0	0	0	0		Living	
49	5/24	5/27	6/14	0	0	0	+	+	7½		Pneumonia; rt. spl. ima intact
50	5/26	6/4	6/12	74	3	+++	+	++	5¾		Both oper. complete; hem. in intestine
51	5/26	6/2	6/10	84	3	+++	++	++	6½	Vomits often	Both oper. complete; hem. in gastric mucosa
52	5/26	6/2	6/10	0	0	0	0	0		Living	
Average				58½	2½				5⅝		

Note: att.—attacks; sever.—severity; dep.—depression; E.E.—electrical excitability; dur.—duration; hem.—hemorrhage; hyper.—hyperemia; br.—branch; spl.—splanchnic; one + slight; two++mild; three+++severe.

TABLE 2

Effect of artificial gastro-intestinal irritation on the severity of parathyroid tetany

DOG	THYPAR.	TOTAL DROPS C. OIL	ONSET	NO. ATT.	SEVER.	DEP.	E. E.	DUR. EXP.	REMARKS	AUTOPSY
			hours					*days*		
2	5/16	12* 8	29	2	+++	++		$5\frac{1}{2}$	Bld. stool; Vomits	Hem. in gastric mucosa
3	5/20	12	48	1	+	+		$2\frac{1}{4}$	Bld. stool	Slight pneumonia; hem. in intestine
4	5/20	20	94	1	+	+		4	Vomits	Pneumonia; hem. in intestine
5	5/22	8	48	1	++	+		$2\frac{1}{4}$	Death in tetany (?)	Hem. in gastric mucosa
6	5/22	8	24	2	+	+		$2\frac{1}{2}$		Pneumonia; hem. in intestine
7	6/17	4	50	1	+	+	+	3	Vomits	Pneumonia; hem. in gastric mucosa
8	6/17	4	0	0	0	+	++	2	Death in tetany (?)	Hem. in intestine
9	6/18	10	47	2	+	++	+	$3\frac{1}{2}$		Pneumonia; hem. in gastric mucosa
10	6/18	6	22	2	++	++	++	6	Vomits blood	Pneumonia; hem. in gastric mucosa
11	6/21	18	0	0	0	+++	++	$9\frac{1}{4}$		Pneumonia; hem. in gastric mucosa
12	6/24	4	27	1	++	+	+	$2\frac{1}{2}$		Pneumonia; hem. in gastric mucosa
13	6/24	16	48	1	++	+++	++	8		Pneumonia; hem. in gastric mucosa
14	6/25	19	0	0	0	++	++	$8\frac{1}{4}$		Pneumonia; slight hem. in intestine
15	6/25	9	54	5	+++	+++	++	8		Pneumonia; hem. in intestine
Average.....			$44\frac{7}{10}$	$1\frac{3}{4}$				5		

* Twelve grains aloes.

Note: c. oil—croton oil; att.—attacks; sever.—severity; dep.—depression; E. E.—electrical excitability: dur. exp.—duration of experiment; hem.—hemorrhage; one +slight; two ++mild; three +++severe.

TABLE 3

Protocol of animal 51, series 1

DATE	HOUR	PROCEDURE	ELECTRICAL REACTION IN MILLIAMPERES			REMARKS
			KCC	ACC	AOC	
5/26	9:00 p.m.	1st op.				
6/4	10:00 a.m.	2nd op.				
6	3:00 p.m.		1.0	2.4		
7	11:15 a.m.		1.8	4.8		
9	8:30 p.m.		1.8	2.8		
16	11:00 a.m.	3rd op.				
	5:00 p.m.		0.8	1.6		
17	10:00 a.m.		0.8	1.2		
	5:00 p.m.		0.6	1.0		Vomits frequently
18	9:00 a.m.		0.4	0.6		Vomits frequently
	5:00 p.m.		0.4	0.6	0.6	
19	10:00 a.m.		0.4	0.5		Vomits frequently
	5:00 p.m.					Slight tremors
	8:00 p.m.					Tremor all evening but not severe
	11:15 p.m.					Mild spastic tetany, particularly in forelimbs
20	3:30 a.m.					Mild spastic and clonic tetany all limbs
	10:00 a.m.					Tetany continues, occasionally severe
	2:00 p.m.					Mild tetany continues
	3:30 p.m.					Tetany gone, tremors present
	5:00 p.m.					Mild tetany again
	9:00 p.m.					Mild tetany, occasionally severe
21	9:00 a.m.					Tremors
	10:00 a.m.					Mild tetany, at times severe
	1:00 p.m.					Mild tetany continues
	5:00 p.m.					Mild tetany has continued all afternoon frequently being severe
	9:00 p.m.					Depressed
22	10:00 a.m.					Depressed
	8:00 p.m.					Found dead

Autopsy:

1. No pneumonia
2. Primary and secondary operations had been complete
3. Slight petechial hemorrhages in gastric mucosa
4. Other organs normal

TABLE 4

Protocol of animal 15, series 2

DATE	HOUR	PROCEDURE	ELECTRICAL REACTION IN MILLIAMPERES			REMARKS
			KCC	ACC	AOC	
6/21	5:00 p.m.		0.5	1.2		
23	10:00 a.m.		0.8	1.0		
25	9:00 a.m.		0.4	1.2	0.8	
	11:00 a.m.	Thy-par				
	5:00 p.m.	2d C. oil				
	8:00 p.m.		0.4	0.8	0.8	
26	9:00 a.m.	2d C. oil				Slight tremors
	12:00 m.					Mild tremors
27	9:00 a.m.	2d C. oil				
	5:00 p.m.					Severe tetany
	10:00 p.m.					Quiet
28	9:00 a.m.	3d C. oil				Severe tetany
	9:00 p.m.					Tetany
29	9:00 a.m.					Severe tremors
	8:00 p.m.					Severe tetany
30	1:00 a.m.					Mild tetany
	10:00 p.m.					Alternating severe tremors and mild tetany
	4:00 p.m.					Severe tremors. Depressed
	9:00 p.m.					Slight tetany
7/1	9:00 a.m.					Depressed
	12:30 p.m.					Mild tetany
	10:30 p.m.					Mild tetany with intervals of depression
2	8:30 a.m.					Depressed. Occasional tremors
3	9:00 a.m.					Very depressed
	1:00 p.m.					Found dead

Autopsy:

1. Extensive pneumonia involving both lungs
2. Hyperemic gastric mucosa with petechial hemorrhages throughout small intestine.
3. Other organs normal

DISCUSSION

In the first table it will be seen that there is no relationship between the dogs which showed tetany and those in which the primary and secondary operations were incomplete. Thus in dogs 40 and 49 the opera-

tions were incomplete and the dogs showed no tetany, while in dogs 36, 38, 50 and 51 the operations were complete and yet the dogs showed tetany. Similarly in the second series there seems to be no relationship between the amount of irritation produced and the onset, severity or number of attacks of tetany. Comparison of the two series shows further that although the average length of time before the onset of tetany was somewhat shorter in the second series and the average length of life a little less, the difference is not enough to be significant especially in view of the fact that the severity of the tetany was somewhat greater in the first series.

The vomiting recorded in the protocol in table 3 and observed also in one other dog in the first series is rather interesting. Carlson and Jacobson observed that in parathyroid tetany "the vomiting is frequently severe and persistent, even in subjects taking no food." Since it occurs in animals with both the vagi and the splanchnics sectioned, one must conclude that possibly this vomiting is of central origin. Subcutaneous morphine-atropine injections preparatory to anesthetization for the tertiary operation was observed to have a similar effect in these animals.

Carlson and Jacobson also called attention to the groaning with evidences of abdominal tenderness so frequently seen in parathyroid tetany dogs. This was shown in our first series by no. 33 especially which was observed to groan continually and although autopsy showed a very small twig of the left splanchnic to be intact, it hardly seems probable that this groaning is due to stimulation of splanchnic or vagus afferent endings.

The results obtained in this work, then, have been entirely negative. The vagi and splanchnics are of no importance in parathyroid tetany in dogs. To reason from this that these nerves are of no importance in tetanies associated with gastro-intestinal disorders is, of course, unsound. However, the present finding does not support the theory of a nervous relationship between the two conditions, but rather lends favor to the view of a chemical relationship.

CONCLUSIONS

1. Double vagotomy and splanchnectomy have no influence upon the onset and course of tetania parathyropriva.

2. Artificial gastro-intestinal irritation has no influence upon the onset and course of tetania parathyropriva.

The above research was done at the suggestion of Dr. A. B. Luckhardt and it is a pleasue here to record my gratitude for his constant interest and helpfulness. The technique was developed by Mr. Raymond Householder in a series of preliminary experiments, for which I am greatly indebted.

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